







## PREFACE TO SECOND EDITION

THE first edition was prepared by the senior author as a collection of methods for inorganic colorimetric analysis. No attempt was made to treat the subject comprehensively, or to include organic and biological applications, and nephelometric methods. The importance of developments in those fields, as well as in the field of colorimetry, since the first edition warrants the much more inclusive treatment of the subject which has been made in this edition. In connection with a more complete survey, the discussion of apparatus has been greatly amplified.

In Volume I the general subject of colorimetry is covered, together with inorganic determinations. Volume II contains organic, biological and miscellaneous methods. A sharp distinction is impossible, because many biological methods, as for example those for phosphorus, naturally fit into the first volume.

Although there is a sharp distinction in technique between colorimetric and nephelometric methods, no such sharp distinction can be made between the solutions to be examined by the two techniques. Lead sulfide, if sufficiently colloidal, is suitable for colorimetric estimation. Standardized so that the particles were larger, it would be suitable for nephelometric estimation. In the intermediate range the errors by either method may be large. The nephelometric and turbidimetric methods included are well known and seem logically to supplement colorimetric methods.

The possibilities of the methods are intriguing. As an example, phosphorus, manganese, nickel, titanium, combined carbon, and several other elements can be determined in a few minutes in iron or steel. The applications to rapid control methods in industry have barely been touched.

With the breadth of application of chemical methods today, no one can be familiar with all of the possible applications of a given method. A method totally unsatisfactory for one purpose may be ideally suited to another, because of variations in the nature and source of samples. A method giving the maximum possible accuracy will be desired for one purpose and involve unnecessary work for another. Therefore, having to choose between the selection of a limited number of methods or of completeness, we have elected completeness of both methods and references, with notations of contradictory results where such have been published. The compromise may prove reasonably satisfactory to both those desiring

critical evaluation and those desiring completeness. Many references are therefore included which may be referred to only in passing. This is particularly true in the chapters on apparatus where the wide ramifications of design of equipment permit description of only the most important, with brief reference to the many varied designs. Additional work to increase the accuracy of many of the methods since the first edition makes such methods more dependable and reliable.

Acknowledgment is made of assistance from specialized texts which have appeared since the first edition, in particular *The Determination of Hydrogen Ions*, Clark, 3rd edition, 1928; *Blood Chemistry Colorimetric Methods*, Stone, 2nd edition, 1925; *Standard Methods of Analysis of Water and Sewage*, American Public Health Association, 6th edition, 1925; *Hydrogen Ion Concentration*, Michaelis, 2nd edition, 1926; *Indicators*, Kolthoff and Furman, 1926; and *Photometric Chemical Analysis*, Yoe, Vol. I, Colorimetry, 1928, Vol. II, Nephelometry, 1929. The latter two have served as particularly valuable check lists of references.

In addition to those acknowledged in the first edition, cuts have been loaned by the Bausch & Lomb Optical Co., La Motte Chemical Products Co., Pfaltz and Bauer, Holmes I. Mettee, Palo-Myers, Inc., American Instrument Co., Emil Greiner Co., R. P. Cargille, Mines Safety Appliance Co., Fisher Scientific Co., Rascher and Betzold, Inc., The Tintometer, Ltd., Hellige, Inc., Carl Zeiss, Inc., E. Leitz, Inc., and others.

We also wish to express our appreciation for the assistance of Beatrice F. Grey in preparation of the manuscript and of Helen C. McBride in reading proof.

We trust that this more comprehensive edition may receive as favorable a reception in its wider field as was accorded the first edition in its limited field.

FOSTER DEE SNELL  
CORNELIA T. SNELL

Brooklyn, N. Y.

## PREFACE TO FIRST EDITION

IT HAS been the endeavor of the author so far as is possible to avoid needless repetition of material. Therefore all points as to theory, apparatus and its use, and calculation of results, which will apply to practically every method given, have been gathered into introductory chapters. From this it will be seen that a knowledge of these chapters is necessary to the success of anyone except an experienced operator in the use of the methods of determination given on the succeeding pages. It has been the endeavor of the author to mention that a particular type of determination was not applicable to those cases where the choice of methods is more limited than usual.

This book is, in essence, an attempt to combine in one volume for ready reference all the inorganic colorimetric methods which experience has shown to be at all practical. Much material will be found here which has never been in print outside of technical journals, as well as the standard methods which may be found by reference to any complete work on chemical analysis. In going over the material, it has been evident to the author that little has been done with regard to the determination of the range of greatest accuracy of many of the methods. Whenever such information is available it has been incorporated, so that the operator may know what degree of accuracy he may expect after he knows the approximate amount of test substance contained in the sample.

It is hoped that this book will furnish a source to which students may refer for a knowledge of the colorimetric methods practical for use, and a source to which practical workers may refer for information as to the methods of performing the particular determinations in which they are interested. In all cases weights of substances to be dissolved as standard have been given to 4 places, although that by no means implies that absolute accuracy is necessary in the last 2 places. Giving these accurate weights will, however, show the operator the direction in which the weight should tend in these last 2 places and thus can promote greater accuracy without increasing the labor of weighing to any great extent.

The author wishes to acknowledge the assistance rendered him by the Library of the University of the State of New York in placing at his disposal their files of journals and reference works. Acknowledgment is also made of the courtesy of Eimer & Amend, C. J. Tagliabue Co. and

Arthur H. Thomas Co. in the furnishing of cuts. Acknowledgment is made of the assistance of C. A. Tyler and W. H. Pearce in the reading of proof.

All factors have been calculated by the 1918 table of atomic weights.

FOSTER DEE SNELL.

New York, N. Y.

July, 1921.

# CONTENTS

PAGE

## CHAPTER I

COLORIMETRIC METHODS .....	1
Series of Standards—Dilution—Balancing—Duplication—Extent of Use—Development of a Colorimetric Method—Beer's Law—Application of Beer's Law to the Colorimeter—Proposed Modification of Beer's Law—Definition of Terms—Illumination—Standard Sunlight—Quality of Color—Color Intensity—Origin of Methods.	

## CHAPTER II

APPARATUS—COMPARISON WITH SERIES OF LIQUID STANDARDS.....	10
Test Tubes—Walpole Technique—Gillespie Comparator—Bottles—Standard Tubes and Jars—Sealed Tubes—Spot Plate.	

## CHAPTER III

APPARATUS—COMPARISON WITH SOLID STANDARDS.....	19
Lovibond Tintometer—Instruments Similar to the Lovibond Tintometer—Hellige Colorimeter and Related Types—Tint Photometers—Colorimetry Without Standards—Trichromatic Colorimetry—Petroleum Colorimeters—Comparison With Color Plates—Color Glasses—Reported Variations.	

## CHAPTER IV

APPARATUS—DILUTION AND DUPLICATION METHODS.	
Dilution Method—Duplication Method.	

## CHAPTER V

APPARATUS—BALANCING METHOD .....	38
Nessler Tubes—Hehner Cylinders—Campbell-Hurley Colorimeter—Modified Campbell-Hurley Instruments—Schreiner Colorimeter—Duboseq Colorimeter—Modifications of Duboseq Colorimeter—Micro Colorimetry—Stammer Colorimeter—Wedge Type Colorimeters—Wedge Type Bi-Colorimeter—Miscellaneous Wedge Types—Special Prism Type—Photoelectric Colorimeters—Yoe Photoelectric Colorimeter—Special Types and Uses.	

## CHAPTER VI

ARTIFICIAL LIQUID STANDARDS .....	66
Cobalt-Iron-Copper Series—Cobalt-Chromate-Copper Series—Peri Chromate Series—Standard Mixtures—Comparison of Standards—Errors—Systems of Color Recording.	

## CHAPTER VII

ACCURACY .....	73
General Limitations—Limitations of Specific Methods—Typical Sources of Error—Mechanical Errors of the Colorimeter—Optical Errors of the Solu-	

tions—Errors from Varied Light—Errors in Readings—Errors of Dilution—Errors from Varied Temperatures—Errors from Varied Times of Standing—Errors from Variable Quantities of Chemicals Present—Errors from Other Solutes Than the Test Substance Present in the Sample—Unavoidable Error of the Individual Operator—Variable Sensitivity of Range—Variable Size of Colloidal Particles—Dichromatism—Turbidity—Purity of Reagents—Errors in Artificial Standards—Importance of Sources of Error.

## CHAPTER VIII

NEPHELOMETRY, PHOTOMETRY AND TURBIDIMETRY.....	90
Development of Nephelometer—Nephelometric Equipment—Correction Curve for the Nephelometer—Range of Usefulness—Applications—Photometry—Turbidimetry—Ostwald's Law.	

## CHAPTER IX

CALCULATIONS .....	97
Series of Standards—Dilution Method—Duplication Method—Balancing Method.	

## CHAPTER X

CARBON .....	101
Carbon in Steel—Combined Carbon in Iron—Carbon in Steel by Conversion to Carbon Dioxide—Carbon in Organic Matter by Conversion to Carbon Dioxide.	

## CHAPTER XI

CARBON MONOXIDE .....	108
Carbon Monoxide by Hoolamite—Carbon Monoxide by Palladium Chloride—Carbon Monoxide by Hemoglobin and Pyrogallol and Tannic Acids—Carbon Monoxide by Ammoniacal Silver Nitrate Solution.	

## CHAPTER XII

CARBON DIOXIDE .....	117
Carbon Dioxide Between 0.03 Per Cent and 6 Per Cent—Carbon Dioxide by Determination of pH—Traces of Carbon Dioxide—Carbon Dioxide by the Sodium Salt of Phenolphthalein.	

## CHAPTER XIII

CYANIDES .....	125
Cyanides as Ferric Thiocyanate—Cyanides as Prussian Blue—Hydrocyanic Acid as Ammonia by Nessler's Reagent—Cyanides by Picric Acid.	

## CHAPTER XIV

OXYGEN .....	130
Oxygen by Cuprous Chloride—Oxygen by the Starch-Iodine Complex—Oxygen by Adurol—Oxygen by Pyrogallol—Oxygen by Indigo Carmine—Oxidizing Power as Oxygen, by Citric Acid and Ammonium Molybdate—Oxygen by Oxidation of Nitric Oxide.	

# CONTENTS

PAGE

## CHAPTER XV

HYDROGEN PEROXIDE AND OZONE .....	
Hydrogen Peroxide by Oxidation of Ferrous Iron—Hydrogen Peroxide by Ammonium Molybdate—Hydrogen Peroxide by Destruction of Nitrite—Hydrogen Peroxide by Oxidation of Titanium Sulfate—Hydrogen Peroxide by Liberation of Iodine—Ozone by Destruction of Nitrite—Ozone by Liberation of Iodine—Ozone by Fluorescein.	

## CHAPTER XVI

COPPER AND CADMIUM .....	
Copper by Ammonia—Copper in Concentrated Hydrochloric Acid—Copper by Salicylic Acid—Copper by Potassium Ferrocyanide—Copper as the Sulfide—Copper by Potassium Ethyl Xanthate—Copper as the Bromide—Copper by Potassium Iodide—Copper by Potassium Cyanide and Guaiacum—Copper in Water in the Presence of Lead—Copper by Pyridine and Thiocyanate—Copper by Benzidine—Copper by Dimethylglyoxime—Copper by Sodium Diethyldithiocarbamate—Copper by <i>m</i> -Benzaminosemicarbazide—Copper by Diphenylthiocarbazone—Copper by Dithiooxamide After Extraction With Diphenylthiocarbazone—Copper by Piperidinium Piperidyldithioformate—Copper by $\beta$ -Naphthol—Copper by Urobilin—Copper by Comparison of the Color of Zinc Mercurithiocyanate Precipitates—Copper by Sodium Arsenite—Copper by Direct Green B—Copper by Reduced Phenolphthalein—Cadmium as the Sulfide.	

## CHAPTER XVII

MERCURY .....	178
Mercury as the Colloidal Sulfide—Mercury by Potassium Diphenylcarbazone—Mercury by Potassium Iodide and Ammonium Hydroxide—Mercury by Phosphomolybdotungstic Acid or Related Reagents—Mercury by Selenium Sulfide—Mercury Nephelometrically by Strychnine Sulfate and Potassium Iodide.	

## CHAPTER XVIII

LEAD .....	186
Lead as the Sulfide—Lead as the Sulfide in Water—Lead in Urine as the Sulfide—Lead in Cream of Tartar as the Sulfide—Lead as the Chromate—Lead After Precipitation as the Chromate—Lead by <i>s</i> -Diphenylcarbazide—Lead as the Chromate by <i>s</i> -Diphenylcarbazide—Lead by Tetramethyldiaminodiphenylmethane—Lead by Diphenylthiocarbazone (Dithizone)—Lead as the Molybdate—Lead by Hematin—Lead by Sodium Bisulfite—Lead Dioxide by Oxidation of Aniline.	

## CHAPTER XIX

THALLIUM .....	207
Thallium as the Sulfide—Thallium by Liberation of Iodine—Thallium as the Phosphomolybdate,	

# CONTENTS

PAGE

## CHAPTER XX

BISMUTH .....	212
Bismuth as the Iodide—Bismuth by Cinchonine Potassium Iodide—Bismuth by Phosphomolybdotungstic Acid—Bismuth by Thiourea—Bismuth as Iodobismuthate of Quinine—Bismuth by <i>o</i> -Hydroxyquinoline—Bismuth by Potassium Thiocyanate—Bismuth as the Sulfide—Bismuth Nephelometrically by Sodium Stannite.	

## CHAPTER XXI

ARSENIC .....	225
Arsenic by the Gutzeit Method—Electrolytic Gutzeit Method—Modified Electrolytic Gutzeit Method—Arsenic by the Cocaine-Molybdate or Strychnine-Molybdate Reagent—Arsenic by Quinine Arsenomolybdate—Arsenic by Sodium Hypophosphite—Arsenic by Formation of Molybdenum Blue—Arsenic by Stannous Chloride—Arsenic by Silver Nitrate—Arsenic as the Trisulfide—Arsenic by Acid Sodium Thiosulfate—Arsenic by Mercurous Chloride.	

## CHAPTER XXII

ANTIMONY .....	251
Antimony as the Sulfide—Antimony by Reaction With Pyridine and an Iodide—Antimony by Phosphomolybdotungstic Acid.	

## CHAPTER XXIII

TIN .....	256
Tin by Ammonium Molybdate—Tin as the Sulfide—Tin by Cacotheliene.	

## CHAPTER XXIV

ALUMINUM .....	259
Aluminum by Alizarin-S—Aluminum by Formation of a Lake With Aurin Tricarboxylic Acid—Aurin Tricarboxylic Acid in Water Analysis—Aluminum by Hematoxylin—Aluminum by 8-Hydroxyquinoline by Conversion to a Dye—Aluminum by Cupferron—Aluminum by Phosphomolybdotungstic Acid—Aluminum by Quinalizarine—Aluminum by Hydroxymethylanthraquinone—Aluminum by Eriochrome Cyanine.	

## CHAPTER XXV

CHROMIUM .....	274
Chromium as the Chromate—Chromium as the Dichromate—Chromium as Chromic Ion—Chromium in Tanning Liquors—Chromium by Disodium 1,8-Dihydroxynaphthalene-3,6-Disulfonate—Chromium by Diphenylcarbazide—Chromium by Afterchroming Dyed Wool.	

## CHAPTER XXVI

IRON .....	283
Iron by Thiocyanate—Iron by Thioglycolic (Mercaptoacetic) Acid—Ferrous and Ferric Iron by Thiosalicylic Acid—Iron by Salicylic Acid—Iron by Salicylsulfonic Acid—Ferric Iron by 7-Iodo-8-Hydroxyquinoline-5-Sulfonic	



# CONTENTS

xi

PAGE

Acid—Iron by Acetylacetone—Iron by Potassium Ferrocyanide—Ferrous Iron by Potassium Ferrieyanide—Iron as the Sulfide—Iron as the Chloride in Concentrated Hydrochloric Acid—Iron by Dimethylglyoxime—Iron by Pyrocatechol—Iron by Pyramidone—Iron by Alloxantin—Iron by  $\alpha,\alpha'$ -Dipyridyl—Iron by 8-Hydroxyquinoline.

## CHAPTER XXVII

NICKEL .....	313
Nickel by Potassium Thiocarbonate—Nickel by Dimethylglyoxime—Nickel by Potassium Dithiooxalate—Nickel by the Formaldoxime Reagent—Nickel as the Sulfide.	

## CHAPTER XXVIII

COBALT .....	321
Cobalt as the Chloride in Concentrated Hydrochloric Acid—Cobalt by $\alpha$ -Nitroso- $\beta$ -Naphthol—Cobalt as Cobaltamine—Cobalt by Potassium Ferrieyanide in Ammoniacal Solution—Cobalt by Dimethylglyoxime—Cobalt by Ammonium Thiocyanate—Cobalt by Reduction of Arsenophosphotungstic Acid—Cobalt by Oxidation of the Cobalt-Cysteine Complex—Cobalt by Hydrogen Peroxide in Alkaline Solution—Cobalt as the Sulfate—Cobalt as the Sulfide.	

## CHAPTER XXIX

MANGANESE .....	332
Manganese as Permanganate—Manganese by <i>o</i> -Tolidine—Manganese by Tetramethyldiaminodiphenylmethane—Manganese by Benzidine.	

## CHAPTER XXX

ZINC .....	345
Zinc by Resorcinol—Zinc as the Sulfide—Zinc by Potassium Ferrocyanide—Zinc by Diphenylthiocarbazone (Dithizone)—Zinc by Phosphomolybdotungstic Acid—Zinc by Urobilin.	

## CHAPTER XXXI

TITANIUM AND CERIUM .....	355
Titanium by Hydrogen Peroxide—Titanium by Sodium Peroxide Fusion—Titanium by Thymol—Titanium by Salicylic Acid—Titanium by Dihydroxymaleic Acid—Titanium by Ammonium Molybdate—Titanium by Gallic Acid—Cerium by Gallic Acid.	

## CHAPTER XXXII

VANADIUM .....	367
Vanadium in the Absence of Titanium, by Hydrogen Peroxide—Vanadium in the Presence of Titanium, by Hydrogen Peroxide—Vanadium in the Presence of Titanium, Molybdenum and Tungsten, by Strychnine—Vanadium by Diphenylamine—Vanadium by Ammonium Molybdate—Vanadium by Phosphotungstic Acid.	

## CHAPTER XXXIII

TUNGSTEN .....	375
Tungsten as the Colloidal Oxide by Titanium Trichloride—Tungsten as the Colloidal Oxide by Stannous Chloride—Tungsten by Potassium Thiocyanate and a Reducing Agent—Tungsten by Hydroquinone—Tungsten by Rhodamine B.	

## CHAPTER XXXIV

MOLYBDENUM AND RHENIUM .....	380
Molybdenum by Hydrogen Peroxide—Molybdenum as the Sulfide—Molybdenum by Potassium Xanthate—Molybdenum by Potassium Thiocyanate—Molybdenum by Phenylhydrazine—Molybdenum by Tannic Acid—Molybdenum by a Tungstic Acid Reagent—Molybdenum by Sodium Thiosulfate—Rhenium by Potassium Thiocyanate.	

## CHAPTER XXXV

URANIUM .....	394
Uranium by <i>o</i> -Hydroxybenzoic Acid—Uranium by Phenolic Acids—Uranium by Potassium Ferrocyanide.	

## CHAPTER XXXVI

BERYLLIUM .....	398
Beryllium by Curcumin—Beryllium by Aurin Tricarboxylic Acid—Beryllium by Quinalizarine.	

## CHAPTER XXXVII

COLUMBIUM .....	401
Columbium by Reduction of the Fluoride.	

## CHAPTER XXXVIII

GOLD .....	402
Gold as Colloidal Gold—Gold by Mercurous Chloride—Gold by <i>o</i> -Tolidine.	

## CHAPTER XXXIX

SILVER .....	409
Silver as the Chloride—Silver as the Sulfide—Silver by Reduction With Hypo-sulfite.	

## CHAPTER XL

PLATINUM .....	413
Platinum by Reduction to Platinous Chloride—Platinum as the Iodide—Platinum and Palladium by Mercurous Chloride.	

## CHAPTER XLI

RHODIUM .....	423
Rhodium by Stannous Chloride—Rhodium in Hydrochloric Acid.	

# CONTENTS

XIII

PAGE

## CHAPTER XLII

IRIDIUM .....	425
Iridium by Benzidine—Iridium in Hydrochloric Acid.	

## CHAPTER XLIII

PALLADIUM .....	427
Palladium by Nitric Acid—Palladium in Platinum by Potassium Iodide— Palladium by Mercurous Chloride.	

## CHAPTER XLIV

POTASSIUM .....	430
Potassium as the Chloroplatinate by Reduction With Stannous Chloride— Potassium as the Chloroplatinate by Potassium Iodide—Potassium Separated as the Cobaltinitrite—Potassium as the Pierate—Potassium and Sodium by Eosin.	

## CHAPTER XLV

SODIUM .....	439
Sodium Separated as a Complex Uranyl Sodium Acetate—Sodium Separated as Pyroantimonate—Sodium Separated as Sodium Caesium Bismuth Nitrite.	

## CHAPTER XLVI

LITHIUM .....	447
Lithium as the Stearate.	

## CHAPTER XLVII

CALCIUM AND BARIUM .....	449
Calcium as Soap—Calcium by Sodium Sulfuricoleate—Calcium and Mag- nesium as the Ferrocyanide—Calcium by Reduction of the Phosphate With Hydroquinone—Calcium by Reduction of the Phosphate With 1,2,4-Amino- naphthol Sulfonic Acid—Calcium by Reduction of the Phosphate With Stan- nous Chloride—Micro Estimation of Calcium as the Phosphate by Reduc- tion With Stannous Chloride—Calcium as the Oxalate by Ferric Thiocyanate —Calcium by the Phenolic Properties of the 8-Hydroxyquinoline Complex— Calcium by Sodium Tungstate and Titanous Chloride—Calcium as the Nickel Nitrite Complex by Antipyrine—Calcium by the Turbidity as Oxalate— Turbidimetric Approximation of Calcium as the Oxalate—Calcium by Alizarin—Calcium as the Pierolonate—Barium as the Sulfate.	

## CHAPTER XLVIII

MAGNESIUM .....	468
Magnesium as the Phosphate by Reduction With Hydroquinone—Magnesium as the Phosphate by Ferric Thiocyanate—Magnesium by Conversion of the Phosphate to the Phosphomolybdate—Magnesium by the Phenolic Properties of the 8-Hydroxyquinoline Complex—Magnesium by 8-Hydroxyquinoline by	

Development of Color With Iron—Magnesium by Decolorization of 8-Hydroxyquinoline Solution—Magnesium by 8-Hydroxyquinoline by Conversion to a Dye—Magnesium by Ammonium Ferrocyanide—Magnesium by Titan Yellow—Magnesium by Curcumin—Magnesium as the Alizarinate—Magnesium as the Oleate—Magnesium Nephelometrically as the Phosphate.

## CHAPTER XLIX

## PHOSPHORUS ..... 480

Phosphorus as Phosphomolybdate—Phosphorus Separated by Precipitation as Magnesium Ammonium Phosphate—Phosphorus by Molybdenum Sulfide—Phosphorus in the Presence of Silica by Ammonium Molybdate—Phosphorus as the Phosphovanadomolybdate—Phosphorus by Reduction of the Phosphomolybdate—Phosphates in Water by Stannous Chloride Reduction of the Molybdate—Phosphates in Sea Water by Stannous Chloride Reduction of the Molybdate—Phosphate as Silver Phosphate—Phosphate by the Strychnine Reagent—Phosphorus in Cast Iron by the Strychnine Reagent—Phosphate by the Strychnine Reagent as Developed With Ferrocyanide—Phosphate by the Quinine Reagent—Phosphate by Uranium Acetate and Potassium Ferrocyanide—Phosphorus in Combustible Gases—Phosphorus by Silver Nitrate.

## CHAPTER L

## SILICA ..... 517

Silica as the Silicomolybdate—Silica and Phosphorus by Ammonium Molybdate—Phosphate and Silica Together as the Molybdate by Hydroquinone Reduction—Silica by Reduction of the Silicomolybdate—Silicic Acid by Pyrrol.

## CHAPTER LI

## BORON ..... 526

Boric Acid by Curcumin—Boric Acid by Turmeric Paper—Boron by Comparison of the Stains Produced With Turmeric.

## CHAPTER LII

## CHLORIDES ..... 531

Chlorides Nephelometrically as Silver Chloride—Chlorides by Determination of Silver in Silver Chloride—Chlorides Nephelometrically by Determination of Excess Silver—Turbidimetric Estimation of Chloride—Chlorides Indirectly by Silver Chromate—Chlorides Through Silver Chromate by Development as the Iodide.

## CHAPTER LIII

## CHLORINE AND CHLORAMINE \* ..... 538

Chlorine by *o*-Tolidine—Chlorine by Benzidine—Chlorine by Dimethyl-*p*-phenylenediamine—Chlorine by Starch-Iodide Solution—Chloramine by Nessler's Reagent—Chloramine by *o*-Tolidine.

# CONTENTS

PAGE

## CHAPTER LIV

BROMIDES .....	544
Bromides by Chlorine-Water—Bromides by Schiff's Reagent—Bromides by Fuchsin—Bromides by Gold Chloride—Bromides by Extraction as Bromine—Bromides by Fluorescein—Bromides by Phenol Red.	

## CHAPTER LV

IODINE .....	551
Iodine by Solvent Extraction—Iodine as Silver Iodide by Conversion to Silver Sulfide—Iodine by Palladous Chloride—Iodine in the Presence of Bromine by Carbon Bisulfide Extraction—Iodides by Bromine-Water—Iodides and Iodates by <i>o</i> -Tolidine—Iodide by Mercurous Chloride—Iodide in Iodized Salt—Iodine by the Starch-Iodine Reaction.	

## CHAPTER LVI

FLUORIDES .....	571
Fluorides by Bleaching an Oxidized Titanium Solution—Fluorides by Bleaching Ferric Thiocyanate—Fluorides by Bleaching Ferric Acetylacetone—Fluorides by Reaction With Alizarin Sodium Sulfonate and Zirconium Nitrate—Fluorides by Zirconium Oxychloride and 1, 2, 4-Trihydroxyanthraquinone—Fluorides by Zirconium Nitrate and 1, 2, 5, 8-Tetrahydroxyanthraquinone—Fluorides by Estimation of Lead Sulfide.	

## CHAPTER LVII

CHLORATES AND PERCHLORATES .....	588
Chlorates by Aniline Hydrochloride—Chlorates by Ammonium Thiocyanate—Perchlorates by Methylene Blue—Perchlorates by Nitrosodimethylaniline.	

## CHAPTER LVIII

SULFIDES .....	593
Sulfides by <i>p</i> -Aminodimethylaniline—Hydrogen Sulfide as Colloidal Lead Sulfide—Sulfides as Arsenious Sulfide—Sulfur by Evolution as Hydrogen Sulfide—Sulfur in Petroleum Distillates—Hydrogen Sulfide as Sulfate—Carbon Bisulfide by Evolution as Hydrogen Sulfide—Carbon Bisulfide as Copper Xanthate—Carbon Bisulfide by Copper and Diethylamine—Sulfur Monochloride by Ammonia.	

## CHAPTER LIX

SELENIUM, TELLURIUM AND THIOCYANATES .....	604
Selenium by Potassium Iodide—Selenious Acid by Pyrrol—Selenium by Sodium Hyposulfite—Selenium by Reduction With Hydroxylamine—Selenium by Mercurous Chloride—Tellurium by Mercurous Chloride—Thiocyanate as Ferric Thiocyanate—Thiocyanate as Copper Pyridine Thiocyanate.	

## CHAPTER LX

SULFITES AND SULFATES .....	611
Sulfur Turbidimetrically as Suspended Barium Sulfate—Sulfate by the Tyndall Effect—Sulfate Turbidimetrically in Water—Sulfate Nephelometrically as Barium Sulfate—Sulfate Separated as Benzidine Sulfate by Iodine and Potassium Iodide—Sulfate Separated as Benzidine Sulfate by Furfurol—Sulfate Separated as Benzidine Sulfate, Diazotized and Coupled—Sulfate by Liberation of Chromate and Estimation With Diphenylcarbazide—Sulfate as the Equivalent Alkali Chromate—Sulfate by Lead Sulfide—Sulfur Dioxide as Sulfate—Sulfur Dioxide by Reduction of Phosphomolybdic Acid.	

## CHAPTER LXI

NITRATES .....	629
Nitrates by Phenoldisulfonic Acid—Nitrates by Brucine—Nitrates by Strychnine and Sulfuric Acid—Nitrates by Diphenylamine on a Spot Plate—Nitrates by Diphenylamine or Diphenylbenzidine in Sulfuric Acid—Nitrates by Diphenylamine Sulfonic Acid—Nitrates by Pyrogallol—Nitrates by Reduction to Ammonia—Nitric Acid in Sulfuric Acid by Estimation as Picric Acid.	

## CHAPTER LXII

NITRITES .....	644
Nitrites by Sulfanilic Acid and $\alpha$ -Naphthylamine—Nitrites by Dimethyl- $\alpha$ -Naphthylamine—Nitrites by $\alpha$ -Naphthylamine and Tartaric Acid—Nitrites by $\alpha$ -Naphthylamine and $\beta$ -Naphthylamine-6, 8-Disulfonic Acid—Nitrites by Dimethylaniline—Nitrites by Metaphenylenediamine—Nitrites by Antipyrine—Nitrites by Zinc Iodide and Starch Solution—Nitrites by Conversion to Nitrates.	

## CHAPTER LXIII

AMMONIA .....	649
Nitrogen as Ammonia by Nessler's Reagent—Ammonia Nephelometrically by a Modified Nessler's Reagent—Ammonia by Phenol and Sodium Hypochlorite—Ammonia by Silver Nitrate and Tannin.	

## CHAPTER LXIV

HYDROGEN-ION—GENERAL .....	660
Theory—Absorption Curves—Interpretation—Colorimetric Principles—Principle of Buffer Solutions—Sources of Error.	

## CHAPTER LXV

HYDROGEN-ION—BUFFERS AND INDICATORS .....	671
Simple Standard Buffer—Clark and Lubs Buffer Solutions—Kolthoff and Vleeschhouwer Buffer Solutions—Buffer for a Wide Range—Manipulation—Buffer Mixtures—Indicator Solutions—pH of Indicator Solutions—Universal Indicator—Glass Standards.	

# CONTENTS

XVII

PAGE

## CHAPTER LXVI

HYDROGEN-ION—SAMPLES .....	687
Water and Clear Liquids—Sea Water—Acetone-Water Mixtures—Sugar Solutions—Molasses—Sulfonated Oil—Soil—Milk—Brewery Products—Bread—Meat Extract—Crackers—Gelatin Solution—Glue—Soap Solutions—Sulfated Fatty Alcohols—Textile Assistants—Unbuffered Solutions—Nutrient Agar—Biological Samples—Urine—Gastric Contents—Cerebrospinal Fluid—Blood, Serum or Plasma—Cells and Tissues.	

## CHAPTER LXVII

HYDROGEN-ION—METHODS OF DETERMINATION .....	697
Comparison With Standard Buffers—Estimation Without Buffers—Balancing Method—Pulfrich Photometer—Blood, Serum or Plasma—Colored Solutions—Comparisons With Test Strips—Comparison on a Spot Plate.	





# TABLES

TABLE

PAGE

1—Calibration of "Pyrotannic Detector" in Terms of Per Cent of Carbon Monoxide in Ethylene .....	115
2—Strength of Sodium Salt of Phenolphthalein for Estimation of Carbon Dioxide .....	122
3—Colors Developed by Copper, Potassium Iodide-Starch .....	158
4—Charges for Separation of Platinum by Fire Assaying .....	413
5—Standards for Chlorine with <i>o</i> -Tolidine .....	540
6—Turbidimetric Sulfur Table. For Use With Jackson's Candle Turbidimeter. Sulfur and SO <sub>2</sub> Contained in 100 cc. Precipitated .....	614
7—Permanent Platinum-Cobalt Standards for Ammonia Nitrogen by Nessler's Reagent .....	656
8—Interrelation of pH, C <sub>H</sub> and C <sub>OH</sub> .....	665
9—pH Values for 0.1 <i>N</i> Solutions, at 20°, Rounded to the First Decimal ...	666
10—Salt Errors With Clark and Lubs Indicators .....	668
11—Buffer Mixtures for pH 1.0-2.2 .....	674
12—Buffer Mixtures for pH 2.2-3.8 .....	675
13—Buffer Mixtures for pH 4.0-6.2 .....	675
14—Buffer Mixtures for pH 5.8-8.0 .....	675
15—Buffer Mixtures for pH 7.8-10.0 .....	676
16—Buffer Mixtures for pH 9.2-11.0 .....	676
17—Buffer Mixtures for pH 11.0-12.0 .....	676
18—Buffer Mixtures for pH 2.2-8.0 .....	677
19—Monochromatic Indicators of Michaelis .....	678
20—Indicators of Sorensen .....	679
21—Indicators of Clark and Lubs, Cohen, and LaMotte Chemical Products Company .....	681
22—Indicators for High pH Values .....	682
23—Correction Factors for Colorimetric Determination of Hydrogen-Ion Concentration of Milk and Whey by Dilution Method .....	692
24—Indicators for Use With Gillespie-Hatfield Method .....	698
25—Bromophenol Blue Standards .....	699
26—Methyl Red Standards .....	700
27—Bromocresol Purple Standards .....	700
28—Bromothymol Blue Standards .....	700
29—Phenol Red Standards .....	701
30—Cresol Red Standards .....	701
31—Thymol Blue Standards .....	701
32—Readings for Balancing Type Colorimeter .....	704
33—Readings for Balancing Type Colorimeter When Standard Column is 15 mm. ....	706

TABLE	PAGE
34—Readings for Balancing Type Colorimeter When Standard Column is 10 mm. ....	708
35—Readings for Balancing Type Colorimeter When Standard Column is 20 mm. ....	709
36—Wave Lengths for Reading Indicators by Pulfrich Photometer.....	711
37—Table of pH Values at 20° and 38° at 0.05 Intervals, With Corresponding Amounts of 0.0075 Per Cent Phenol Red and 0.01 N NaOH or 0.0001 N HCl .....	713

# ILLUSTRATIONS

FIGURE	PAGE
1—Turbidimeter and Nephelometer for Comparison of Series of Standards....	10
2—Comparator for Hydrogen-Ion Measurements, Interior Painted Black....	11
3—Eggertz Tubes .....	13
4—Julian Tubes .....	13
5—Printed Page Observed from the Top of Two Nessler Tubes.....	14
6—Block Comparator for Series of Liquid Standards.....	14
7—Roulette Comparator for Series of Liquid Standards.....	15
8—Cooledge Hydrogen-Ion Color Comparator .....	16
9—Sliding Comparator Designed for Sugar Work.....	16
10—S. D. C. Colorimeter for pH Determination.....	17
11—Lovibond Tintometer .....	18
12—Simple Form of Lovibond Tintometer.....	18
13—Sample in Horizontal Lovibond Tintometer Using Artificial Light.....	18
14—Lovibond Tintometer for pH Determination.....	18
15—Vertical Form of Lovibond Tintometer for Use With Artificial Light.....	19
16—Hellige Laboratory Comparator .....	23
17—Schematic Diagram of Hellige Comparator.....	24
18—Hellige Comparator With Illuminator .....	24
19—Simple Hellige Comparator .....	24
20—Hellige Pocket Comparator .....	24
21—Ives Tint Photometer .....	25
22—Pulfrich Photometer .....	26
23—Saybolt Universal Chromometer .....	28
24—Conversion of Lovibond to Stammer Readings.....	29
25—Tag-Robinson Colorimeter .....	30
26—Union Colorimeter .....	31
27—Glass Standard in Place on Duboseq Colorimeter.....	32
28—Glass Standards for Use With Duboseq Colorimeter.....	32
29—Dilution Colorimeters .....	36
30—Color Camera .....	36
31—Hehner Cylinders .....	39
32—Campbell-Hurley Colorimeter .....	39
33—Schematic Diagram of Campbell-Hurley Colorimeter.....	40
34—Schreiner Colorimeter .....	42
35—Standard Duboseq Colorimeter .....	43
36—Schematic Diagram of Duboseq Colorimeter .....	44
37—Direct-Reading Duboseq Colorimeter .....	44
38—Duboseq Colorimeter With Armored Cups, for Dilute Solutions.....	45
39—Duboseq Colorimeter With Magnifier Scale Which Can Be Read from Observing Position .....	45
40—The Vim-Sheftel Colorimeter .....	47

FIGURE	PAGE
41—Colorimeter Reading Directly as Ratio of Sample and Standard.....	48
42—Diagrammatic Arrangements of Duboseq Type of Hydrogen-Ion Colorimeter.....	49
43—Cell of Standard of Constant Depth for Duboseq Colorimeter.....	49
44—Three Forms of Use of Universal Colorimeter.....	50
45—Micro Duboseq Colorimeter.....	51
46—Stammer Colorimeter.....	52
47—Schematic Diagram of Stammer Colorimeter.....	52
48—Wedge-Type Colorimeter.....	53
49—Colorimeter Wedges.....	53
50—Double Wedge for Standard in pH Determination.....	53
51—Special Prism Type of Colorimeter.....	56
52—Yoe Photoelectric Colorimeter.....	59
53—Diagram of Yoe Photoelectric Colorimeter.....	59
54—Tube and Adapters for Yoe Photoelectric Colorimeter.....	60
55—Two Types of Wesson Colorimeter Used in Examination of Vegetable Oils.....	
56—Colorimeter Lamp for Attachment Directly to Colorimeter.....	77
57—Duboseq Colorimeter With Nephelometer Attachment.....	90
58—Colorimeter Cup. Nephelometer Cup.....	91
59—Kober Nephelometer.....	92
60—Burgess-Parr Turbidimeter.....	94
61—Apparatus for Decomposition of Sample to Determine Carbon and Nitrogen.....	105
62—Apparatus for Estimation of Carbon Dioxide in Mixed Gases.....	106
63—Carbon Monoxide Detector.....	108
64—Diagram of Carbon Monoxide Detector.....	109
65—Apparatus for Fractionation of Carbon Monoxide from Ethylene.....	113
66—Apparatus for Collecting Carbon Dioxide Over the Sodium Salt of Phenolphthalein.....	123
67—Leiboff Apparatus Applied to Hydrolysis of Hydrocyanic Acid.....	128
68—Apparatus for the Determination of Dissolved Oxygen.....	131
69—Apparatus for Estimation of Oxygen by Starch-Iodine Complex.....	134
70—Micro Bomb for Decomposition of Organic Samples.....	229
71—Apparatus for Distillation of Arsenic.....	233
72—Sanger-Black-Gutzeit Apparatus for Arsenic Determination.....	237
73—Electrolytic Gutzeit Apparatus.....	240
74—Modified Electrolytic Gutzeit Apparatus.....	240
75—Apparatus for Absorption of Arsine in Silver Nitrate Solution.....	248
76—Apparatus for Development of Aurin Tricarboxylic Acid Lake.....	264
77—Typical Calibration Curve of Aluminum Aurin Tricarboxylate Against Artificial Standard.....	264
78—Apparatus for Solvent Extraction of Iron from Aqueous Solution.....	287
79—Apparatus for Evolution of Phosphine and Combustion to Phosphoric Acid.....	495
80—Apparatus for Ashing Samples for Iodine Determination.....	553
81—Diagram of Apparatus for Combustion of Samples of Vegetables for Iodine Determination.....	555
82—Apparatus for Distillation of Iodine from Samples.....	561
83—Correction for NO <sub>2</sub> , SO <sub>4</sub> , Cl, BO <sub>3</sub> and PO <sub>4</sub> in Natural Waters for Fluoride Determination.....	575

## FIGURE

84—Amounts of Fluorides in Mg. Equivalent to Iron Color Destroyed.....	576
85—Details of Evolution Apparatus for Fluorine.....	578
86—Evolution Apparatus for Fluorine .....	579
87—Apparatus for Separation of Traces of Fluorides.....	586
88—Apparatus for Evolution of Sulfides With Hydrogen.....	599
89—Apparatus for Evolution of Sulfides With Nitrogen.....	599
90—Calibration Curve for Betz-Hellige Turbidimeter 0 to 5 p.p.m. of Sulfate...	616
91—Calibration Curve for Betz-Hellige Turbidimeter 0 to 15 p.p.m. of Sulfate..	616
92—Calibration Curve for Betz-Hellige Turbidimeter 0 to 50 p.p.m. of Sulfate..	616
93—Calibration Curve for Betz-Hellige Turbidimeter 0 to 100 p.p.m. of Sulfate.	616
94—Betz-Hellige Turbidimeter for Estimation of Sulfate by the Tyndall Effect	617
95—Observation Fields of Betz-Hellige Turbidimeter.....	618
96—Transmittancy Curve for Bromothymol Blue .....	662
97—Paraffined Bottle With Buret and Soda-Lime Guard Tube for Standard Alkali Solution .....	673
98—Hydrogen-Ion Concentration Ranges (pH) and Color Changes of Indicators	683
99—pH Range of Common Indicators .....	684
100—Slide Comparator for Series of Liquid Standards.....	685
101—Hellige Capillary Comparator .....	686
102—Apparatus for Estimation of pH in Unbuffered Solutions.....	694
103—Apparatus for Handling Spinal Fluid in pH Determination.....	696
104—Series of Liquid Color Standards.....	699
105—Modified Duboseq Colorimeter for pH Determination.....	702
106—Duboseq Type of Hydrogen-Ion Colorimeter.....	703
107—Slide Comparator for pH Determination, With Built-in Illuminating System	712
108—British Drug House Capillary Colorimeter.....	714
109—Wulff Colorimeter for pH Determination .....	716



## CHAPTER I

### COLORIMETRIC METHODS

COLORIMETRIC methods of analysis consist of treating a solution of a substance with a reagent in such a way as to produce a color which is proportional in intensity to the amount of the substance present in the solution. The methods are applicable to the determination of many metals, radicals and organic compounds. The unknown is spoken of in this general discussion as the test substance. The color having been produced, the solution containing an unknown amount of test substance is compared with a standard solution by one of four methods.

**Series of Standards**—The sample, diluted to a definite volume, is compared with a series of standards of the same volume, the amount of test substance in which is known. The value of the unknown is taken to be that of the standard to which it conforms most nearly, or estimated from that standard. In this way the amount of test substance present is obtained without calculation since, if the volume and color of the unknown and the standard are the same, their contents of test substance will be identical.

**Dilution**—The standard and sample are placed in similar graduated tubes and the darker diluted until its color when observed horizontally through the tube after mixing, is the same as that of the other. When this point is reached each unit volume of one solution must contain the same amount of test substance as each unit volume of the other and the amount in the unknown is to the amount in the known directly as their volumes.

**Balancing**—A sample solution is placed in a flat-bottom graduated tube and a standard solution added to a similar tube, until the color intensities, when observed through the lengths of the columns of liquid, are identical. The amount of test substance in each tube will then be the same, and since the amount per unit volume in the standard is known, the total amount in the standard may be calculated. This is identical with the

amount in the sample. If the tubes are not of the same cross-section, the amount of test substance per unit of cross section is the same, and the concentrations are to each other inversely as the depths of solution. More briefly, their concentrations are inversely proportional to their depths. This is the method employed by the usual instruments.

**Duplication**—The sample is made up to a definite volume, and nearly that volume of water in a similar container is treated with the same reagents for bringing out the color of the solution, as were used with the sample. A concentrated solution of standard is then added to the blank from a buret, drop by drop when the end point is near. The volume of the blank is then brought up by addition of more water until the two colors and volumes are identical. The amount of standard used in making the duplicate is a measure of the amount of test substance in the sample.

**Extent of Use**—The colorimeter is coming into use more and more in practical analysis because it answers the demand for speed. Colorimetric methods often give results in 5 minutes to 1 hour from the time the determination is begun, which is in many cases less than half the time in which similar determinations could be made by other methods. It may be said for colorimetry in general that its methods are rapid and reasonably accurate.

A broad field of usefulness of colorimetry is the determination of impurities in substances easily soluble in water, alkali or acid. The methods are very delicate and accurate, many being so delicate as to detect one part in one hundred million parts of solution. They are seldom such as to permit the determination of quantities greater than one per cent without resorting to aliquot parts. The drawback to the use of a small aliquot is that the factor for determining the final result multiplies the error made in the comparison by the size of the factor.

**Development of a Colorimetric Method**—In general in the development of a new colorimetric method, only the method by a series of standards can be assumed to be applicable, the standards containing the same amounts of the same reagents as the unknown and prepared at the same time. Beer's law states that the concentration of a solution is directly proportional to its color intensity. Since this is the basis for colorimetry, it merits detailed discussion. If Beer's law is found to be applicable to the reaction, the dilution method can be applied within



limits. If Beer's law holds, the method of balancing is applicable within the limits in which the law has been found to apply. If the color is permanent and if it develops instantaneously, or nearly so, the method of duplication is applicable.

Frequently the product produced from the test substance precipitates above a definite concentration, in which case that sets a limit. The lower limit is usually that at which the color is just perceptible but the accuracy becomes less as this lower limit is approached. In many cases, before precipitation is reached, the substance becomes colloidal rather than dissolved, and sometimes, but not always, the methods become nephelometric under these conditions. In some cases the formation of particles large enough to make the method nephelometric is avoided by addition of a protective colloid such as a water-soluble gum.

**Beer's Law**<sup>1</sup>—Visible light in passing through any medium is absorbed in part. The variation with different media is very great. In passing through water the absorption is small. Deviations in the absorption by 2 columns of water, one 30 mm. deep, the other 40 mm. deep, would be inappreciable for colorimetric purposes. Only very accurate measurements would show them. For the purposes of colorimetric comparison the variation in absorption may be assumed to be entirely due to the test substance dissolved.

A spectroscopic analysis of the light transmitted through a copper sulfate solution would show less absorption in the blue than in the other colors. The eye reports this solution as blue because of the relative absence of other waves. Depending on the composition of the solution yellow waves may or may not be greatly reduced. If there is lessened absorption in the yellow range the blue is changed from pure blue toward green.

While passing through an infinitesimal layer of the solution,  $dl$ , the energy of a given wave length is reduced by a fraction of its intensity,  $I$ . In the next infinitesimal layer a similar fraction of the remaining intensity is absorbed. The decrease of intensity per unit depth of solution,  $I$ , is therefore proportional to the intensity of energy passing through that layer according to the expression

$$(1) \quad \frac{-dI}{dl}$$

Integration of this between the initial intensity and the emergent intensity  $I_2$  gives

---

<sup>1</sup> Beer, *Ann. Physik* (2) 86, 78-88 (1852).

## COLORIMETRIC METHODS

$$(2) \quad -\log_e \frac{I_2}{I_1} =$$

In the final analysis the decline of intensity in an infinitesimal layer is directly proportional to the number of absorbing molecules encountered which will be proportional to the concentration of test substance present, *c*. From (1) this gives

$$(3) \quad \frac{-dI}{dl} = kcI$$

which between the limits  $I_1$  and  $I_2$  gives

$$(4) \quad \log_e \frac{I_2}{I_1} = -kcl$$

The fraction of the intensity,  $\frac{I_2}{I_1}$  which emerges is known as the transmittance and may be represented by  $T$ . Changing the logarithms of base *e* in (4) to those of base 10 and substituting the transmittance gives an expression of Beer's law.

$$(5) \quad -\log T_\lambda = lck_\lambda$$

In this the subscript  $\lambda$  indicates that the values for the terms are only for a given wave length of the radiant energy to which the transmittance  $T_\lambda$  applies.

Similarly (2) gives Lambert's law.

$$(6) \quad -\log T_\lambda = lK_\lambda^1$$

**Application of Beer's Law to the Colorimeter**—Assume that two solutions receive the same intensity of light at a wave length  $\lambda$ . Each contains a test substance to which the absorption constant  $K_\lambda$  applies. The concentration of test substance in the two solutions is different. If we adjust the depth of one solution until the intensity of light at wave length  $\lambda$  is the same as from the other solution, their transmittances have then been made equal.

The values from (5) are then

$$(8) \quad \begin{aligned} -\log T_\lambda &= l_1 c_1 K_\lambda \\ -\log T_\lambda &= l_2 c_2 K_\lambda \end{aligned}$$

from which it follows that

$$(9) \quad l_1 c_1 = l_2 c_2 \text{ and } c_1 : c_2 = l_2 : l_1$$

The balancing colorimeter is a device specifically arranged for adjustment of the intensities of the light transmitted by two solutions until their transmittances are equal. Knowing  $c_1$  and measuring  $l_1$  and  $l_2$ , (9) can be readily solved for  $c_2$ .

This must of necessity be derived on the assumption that light of only a single wave length is used. To visualize the entire range a similar application must be assumed to each wave length. Since, in the absence of dichromatism, the ratios of the different wave lengths remain constant, all of the forms of (7), (8) and (9) would be the same and it follows that in practice (5) is simplified to

$$(10) \quad -\log T = lcK$$

in which now  $T$  represents the transmittance for the solution and  $K$  a constant for the absorbing substance in the solution. Since their relation to each other is constant at all wave lengths they can be determined at any wave length.

When the statement is made that Beer's law holds for a solution it means that the tint of the solution is dependent only on the mass of dissolved solute, independent of dilution. It could be expected to hold exactly only for non-ionizable substances in true solution. On that basis it would seldom hold exactly. As a matter of practice it is observed to hold within the usual limits of observation for the majority of colorimetric methods of analysis.

**Proposed Modification of Beer's Law**—Even when Beer's law holds approximately, there is still the minor factor of the color of different depths of the solvent. It has, therefore, been proposed <sup>2,3</sup> that the simple form

be altered to

$$(11) \quad k) =$$

In this form results could be obtained in more dilute solutions. The factor  $k$  could be determined empirically for each coloring substance by dilution of a solution of low concentration until it matches the solvent. It

<sup>2</sup> Martin Winkler, *Chem.-Ztg.* 56, 86-7 (1932).

<sup>3</sup> Cf. H. Ginsberg, *Z. anorg. allgem. Chem.* 209, 105-12 (1932).

could also be computed from the difference in apparent and actual content of the substance being measured in the standard. The  $k$  is, therefore, an evaluation of the color of the solvent in terms of the developed color. The principal obstacle seems to be accurate evaluation of  $k$ . In limited cases where comparisons are necessarily done at extremely low color intensities, it would improve the degree of accuracy.

**Definition of Terms**—The Colorimetry Committee of the Optical Society of America<sup>4</sup> has attempted to define the terms of colorimetry. So far as these apply to practical colorimetry their definitions follow and have been used.

*Color* is the general name for all sensations arising from the activity of the retina of the eye and its attached nervous mechanisms, this activity being, in nearly every case in the normal individual, a specific response to radiant energy of certain wave lengths and intensities. It is fundamentally psychological and cannot be synonymous with wave length. It may be defined in terms of three fundamental attributes.

*Brilliance* is that attribute of any color in respect of which it may be classed as equivalent to some member of a series of grays ranging between black and white. Synonyms are luminosity, brightness, tint, value and visual brightness.

*Hue* is that attribute of certain colors in respect of which they differ characteristically from the gray of the same brilliance and which permits them to be classed as reddish, yellowish, greenish or bluish.

*Saturation* is that attribute of all colors possessing a hue which determines their degree of difference from a gray of the same brilliance. Synonyms are purity and chroma.

In colorimetry hue and saturation are assumed to have a fixed ratio to each other and brilliance is measured. This is complicated by the use of a mixture of colors, each assumed to meet these conditions.

**Illumination**—This is desirably standard which means the use of artificial light. It is approximated in practice by use of north daylight or some reasonably duplicable source of indirect illumination. The variation in quality of daylight between urban and suburban districts or between seasons is well known and is a source of some errors. It is easily possible that two solutions would compare at different levels when the ratio of red to blue light differed, as between bright and cloudy days.

---

<sup>4</sup> L. T. Troland et. al., *J. Optical Soc. of Amer. Rev. Sci. Instruments* 6, 527-96 (1922).

**Standard Sunlight**—The most accurate <sup>4</sup> standard sunlight is that of Priest <sup>6</sup> in which the radiation from a gas-filled tungsten lamp,<sup>7</sup> operated at a color temperature of 2848° C., is passed through a pair of crossed nicol prisms between which is a quartz plate 0.500 mm. thick with surfaces perpendicular to the optic axis of the crystal. Other methods involve blue glasses or gelatine filters before standard illuminants. The most available is a No. 78 Wrattan photometric filter <sup>8</sup> or a standard cylindrical acetylene flame.<sup>9</sup> A Mazda B tungsten light burning at 1.25 watts per mean horizontal candle power approaches this very closely. Other selectively absorbing glasses are Luckiesh "Trutint" and Corning "Daylite".

**Quality of Color**—Colors as measured by the colorimeter are a measure of that band of visible rays not absorbed by the solution. Similarly, of a solid the color measured is that not absorbed by the solid. Such variations in quality of a color as occur are due to variation in the intensities of the bands which cause the sensation. An iron solution shows red because its transmission is largely of the red end of the spectrum. If more yellow is transmitted by another solution intended for comparison, as may occur by variation in the reagents present or the concentration of iron salt, the hue will become yellowish. Except in a few cases where methods of correction are known, the two can be satisfactorily compared only by use of a special technique. Similarly, if a copper solution contained a nickel salt as impurity, the color would tend toward green, or if it contained a cobalt salt the color would tend toward violet.

The quality of color is usually maintained at a definite standard of distribution of wave lengths by following precautions given in detail with the particular method. These are designed to accomplish the following:

- (1) Insure the same concentration of all reagents in the standard and sample.
- (2) Avoid extreme variation in concentration of test substance between standard and sample.
- (3) Insure absence of all other materials than the test substance which are of themselves colored or will produce a color with the reagents used.

<sup>6</sup> I. G. Priest, *Physical Rev.* 11, 502-4 (1918).

<sup>7</sup> Available from the National Bureau of Standards.

<sup>8</sup> Eastman Kodak Co., Rochester, N. Y.

<sup>9</sup> L. A. Jones, *Trans. of the Illum. Eng. Soc.* 9, 716-28 (1914).

**Color Intensity**—In general in true colorimetry only the apparent effect is measured. As an example <sup>10</sup> a dandelion and a sodium flame have the same apparent color. The dandelion reflects all colors except blue, thus giving rise to a sensation of yellow. The sodium flame emits only yellow of sharply defined wave lengths.

In photometry the analysis of the two would be radically different, in colorimetry they would be the same. In colorimetry the two colors being compared are assumed to differ from each other only in intensity. Conditions are controlled with that end in view. The major case where the two may be radically different yet appear the same, is when artificial standards are used.

**Origin of Methods**—The colorimetric method is a logical development from the estimation by eye of the laboratory analyst. In looking at the permanganate solution resulting from a bismuthate determination of manganese one can almost guess the amount of manganese in the sample. The development of methods which would measure such color where it is reasonably stable might well be expected. The attention which has been devoted to this is clearly indicated by the apparatus developed for the purpose.

Such a development of methods is also logical from the present tendency in qualitative analysis, where a student is expected not only to report the elements present, but whether in large or small amounts. In thus tacitly classifying colorimetric analysis as a glorified qualitative analysis, the best picture of the field is probably obtained. In their individual ranges of application, the methods are usually accurate to better than 5 per cent. They are not to be considered as of the same degree of accuracy as gravimetric or volumetric methods, except for very small amounts. For very small amounts they are frequently much more accurate than other methods.

Classification as a glorified qualitative analysis also gives a fair picture of the possible field of new methods. A new reagent for a qualitative test, such as "aluminon" for aluminum, especially if it gives a color reaction proportional to the amount of the test substance present, would be expected to lead to development from a qualitative test into a method of estimation. There are doubtless hundreds of qualitative tests giving color reactions which are in use in private laboratories for quantitative colorimetric estimation, but of which no details have been published. If one wanted to determine the amount of water in absolute alcohol, could not a

---

<sup>10</sup> C. E. K. Mees, *J. Ind. Eng. Chem.* 13, 729-31 (1921).

tightly stoppered sample be shaken with a few permanganate crystals and the intensity of pink compared quickly with suitable non-alcoholic permanent standards? Probably such a method is in use but unpublished. With very few exceptions qualitative color reactions are potential colorimetric methods of analysis by application of a suitable type of method.

## CHAPTER II

### APPARATUS—COMPARISON WITH SERIES OF LIQUID STANDARDS

AS PREVIOUSLY outlined there are four types of determinations made by colorimetric methods, each of which uses a more or less specialized form of apparatus. The development of specialized apparatus has progressed to such a degree that it is only feasible to describe typical and widely used examples of each kind in detail with brief mention of some of the numerous modifications.

The apparatus varies in complexity from a simple estimation in two

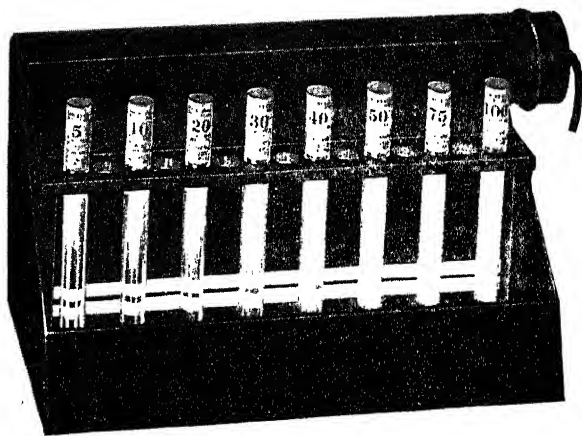


FIG. 1

Turbidimeter and Nephelometer for Comparison of Series of Standards. (Courtesy of R. P. Cargille, New York, N. Y.)

test tubes through the use of calibrated Nessler tubes, simple and complex forms of balancing apparatus to the tintometer and color analyzer. The latter are on the border line of true colorimetric analysis. The complexity of apparatus justified for a determination will vary with the accuracy desired and with the number of determinations to be made.

**Test Tubes**—For the production of nonpermanent standards plain test tubes are often used. The solution may be quickly and conveniently



emptied from these and the breakage loss is not so great as from the use of graduated Nessler tubes or bottles. Even with such simple tubes, proper lighting such as that shown in Figure 1 greatly facilitates comparison.

**Walpole Technique**—If a sample has a natural color it may still be examined by colorimetric methods provided the intensity of the natural

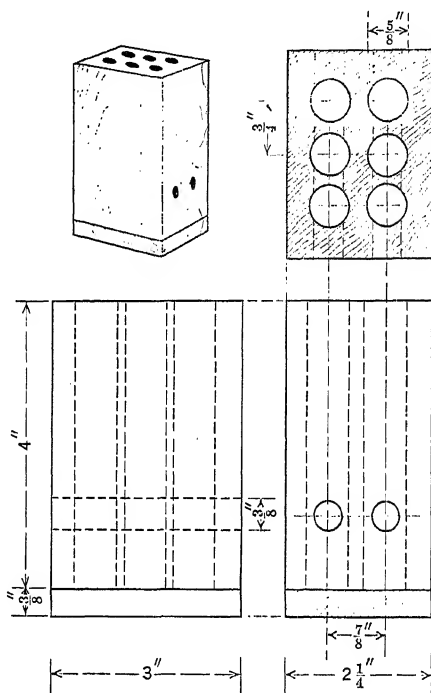


FIG. 2

Comparator for Hydrogen Ion Measurements, Interior Painted Black

color is not excessive. The technique provides for correction of the color of the standard by observing it through a tube of undeveloped sample.

To take a simple illustration, let us assume that oxygen is to be estimated by the pyrogallol method in a tube of a naturally colored sample. The standard does not contain a similar color but the same effect is obtained by looking through a tube of untreated sample and the standard at the same time. To have the thickness of liquid observed with the sample

the same, one looks through the developed sample and through a tube of water.

If A is the developed sample, B the undeveloped sample, C a tube of water, and D one of the series of standards, one observes A and C against B and D. Various standards are used as D until the color of the pair BD matches the pair AC.

**Gillespie Comparator**—As a form of the Walpole technique originated by Mines<sup>1</sup> for application to pH determination, a block comparator is used for comparison of a sample tube with two additive standards. The details of the apparatus are shown in Figure 2.<sup>2</sup> On the left side is the developed sample with one or two tubes of distilled water. On the right side are two standards with a third tube of undeveloped sample if the latter is colored. The principle is generally applicable for tubes of colorimetric standards. Use of quadrangular receptacles is preferable to round tubes.<sup>2a</sup>

A microcomparator has been made from a microscope slide box for similar standards.<sup>3</sup> A pocket colorimeter has been designed which uses a case containing a series of standard tubes.<sup>4</sup> The inside of the box is painted with a contrasting color.

**Bottles**—Where a series of permanent standards is prepared, these are placed in round or square glass bottles. Such a series of standards should be placed in a row, with sufficient space between each pair for a similar bottle. The sample should be treated in a similar bottle and, after dilution to the same volume as that of the standards, placed in various gaps between the standards until a place is found where one standard is higher than the sample and the one on the other side lower, as estimated from the intensities of their colors. The position of the sample relative to these two known quantities can then be estimated.

In some cases when permanent standards are used one bottle may be made to serve as two standards. This is accomplished by the use of a rectangular bottle, twice as long as it is broad. The sample is compared in a similar bottle. If the long way of the sample bottle compares with the long way of the standard the amounts of test substance in the two

---

<sup>1</sup> G. R. Mines, *J. Physiol.* 40, 327 (1910).

<sup>2</sup> Louis J. Gillespie, *J. Amer. Chem. Soc.* 42, 746 (1920).

<sup>2a</sup> Maurice Dérivé, *Ann. chim. anal. appl.* 17, 149-50 (1935).

<sup>3</sup> A. R. Peebles and R. C. Lewis, *J. Am. Med. Assoc.* 70, 679-80 (1918).

<sup>4</sup> Alfred M. Parks, U. S. Patent 977,964 (1910).

are the same. If the narrow way of the sample compares with the long way of the standard the sample contains twice as much test substance as the standard. If the long way of the sample compares with the narrow way of the standard the sample contains half as much test substance as the standard. The use of such bottles serving as two standards presupposes that Beer's law holds and is only justifiable when that can be proved. The color must vary only in intensity, not in quality, with change of concentration. Care should be taken to see that the bottles used in all cases are clear in color, free from flaws, and of uniform size.



FIG. 3  
Eggertz Tubes

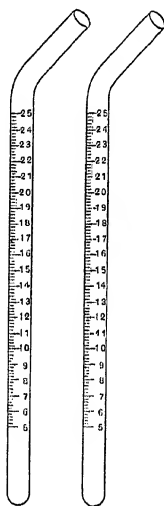


FIG. 4  
Julian Tubes

**Standard Tubes and Jars**—The following are the only standard color comparison tubes recommended as stock sizes by the Committee on Guaranteed Reagents and Standard Apparatus of the American Chemical Society.<sup>5</sup> The specifications for the apparatus are also given.

Eggertz tubes without stoppers in sets of 2 or 4, capacity 30 cc. or 50 cc.

Julian tubes in sets of 2 or 4 graduated to 0.1 cc. from 5 to 30 cc. or from 10 to 50 cc.

Nessler tubes in sets of 6 or 12, American Public Health Association

<sup>5</sup> W. D. Collins, *J. Ind. Eng. Chem.* 14, 655 (1922).

Standard, tall form, clear glass, polished bottoms, calibrated at 50 cc., 100 cc. or 50 and 100 cc. Calibration marks on 50 cc. tubes are from 200 to 250 mm. from the bottom and on the 100 cc. tubes from 275 to 325 mm.

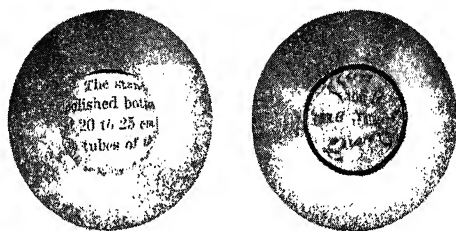


FIG. 5

Printed Page Observed from the Top of Two Nessler Tubes. Left—A Double-Plane Tube. Right—Tube With but One Plane Surface. (Courtesy of Fisher Scientific Co.)

from the bottom. In sets, the highest and lowest marks shall not be more than 6 mm. apart.

Nessler jars in sets of 2 or more, colorless glass, polished bottom, calibrated at 50 cc., 100 cc. or 50 and 100 cc.

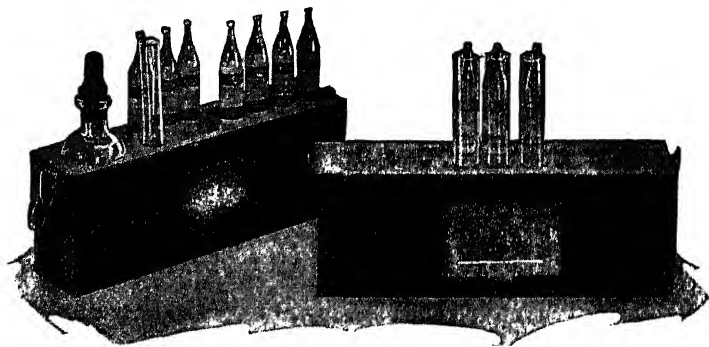


FIG. 6

Block Comparator for Series of Liquid Standards. (Courtesy of La Motte Chemical Product Co.)

Other sizes and types will be available from some manufacturers. The types of Nessler tubes, of which the bottom is polished on both sides and fused on, are preferable as avoiding distortion and inequality of light.

The optical system of the Campbell-Hurley instrument<sup>5a</sup> has been combined with the roulette comparator for sealed tubes to facilitate examination of samples and standards in Nessler tubes.<sup>5b</sup>

**Sealed Tubes**—For specialized purposes permanent standards in sealed tubes are convenient. For hydrogen-ion work these have been developed to a high degree of refinement. The equipment consists of kits

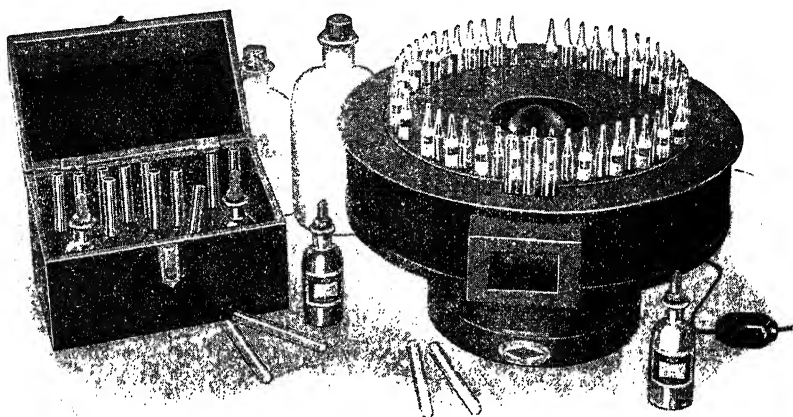


FIG. 7

Roulette Comparator for Series of Liquid Standards. (Courtesy of La Motte Chemical Product Co.)

of prepared standards, or of a rotary apparatus<sup>6</sup> carrying the tubes in which the comparison can be made without removal from the holder.

For rapid comparison of tubes of standards, sliding racks have been designed,<sup>7,8,9,10,11</sup> also a rack with a sliding shield<sup>12</sup> holding the tube to be compared. The Cooledge instrument has 2 racks, each of which holds 12 test tubes. A cord moves the rear rack and a knob on the front of the

<sup>5a</sup> See p. 39.

<sup>5b</sup> John H. Yoe and Thomas B. Crumpler, *Ind. Eng. Chem., Anal. Ed.* **7**, 78 (1935).

<sup>6</sup> H. C. Prinsen-Geerligs, *Arch. Suikerind.* **42**, 189-90 (1934).

<sup>7</sup> J. W. Weir, *J. Lab. Clin. Med.* **3**, 132-3 (1918).

<sup>8</sup> L. H. Cooledge, *J. Ind. Eng. Chem.* **12**, 499 (1920).

<sup>9</sup> G. P. Meade and R. Baus, *Sugar Mfr. and Planter*, **74**, 509 (1925).

<sup>10</sup> S. L. Leiboff, *Ind. Eng. Chem., Anal. Ed.* **2**, 194 (1930).

<sup>11</sup> Werner Ulrich and Theodor Kunzmann, *Chem.-Ztg.* **57**, 18-20 (1933).

<sup>12</sup> J. S. Brotherhood, *J. Am. Med. Assoc.* **64**, 1757 (1915).

colorimeter moves the front rack. Thus a series of samples in one rack may be rapidly compared with a series of standards in the other. A somewhat similar apparatus for use with artificial light has been de-

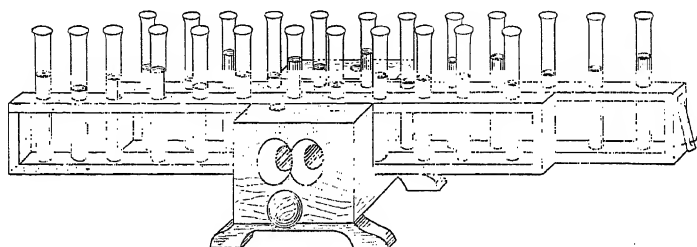


FIG. 8

Cooledge Hydrogen-Ion Color Comparator

scribed.<sup>13</sup> For clinical use a metal frame with a window for lighting two tubes of the set is convenient.<sup>14</sup> A color screen may be interposed.<sup>15</sup> Special sets of liquid standards for electroplating, chlorine and many other purposes are available.

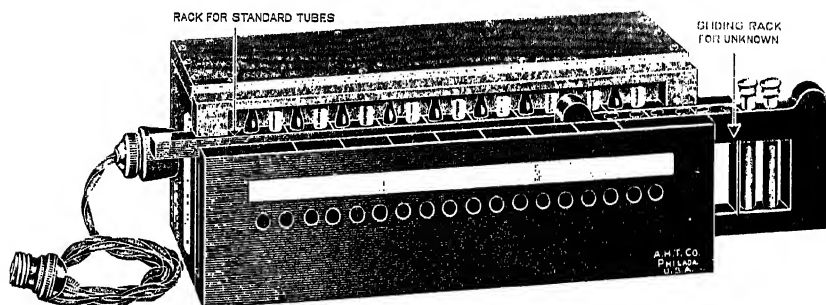


FIG. 9

Sliding Comparator Designed for Sugar Work. (Courtesy of A. H. Thomas Co.)

Comparison of rectangular sealed ampoules with samples, particularly for hydrogen-ion determination is provided in a recent instrument.<sup>16</sup>

<sup>13</sup> C. H. Ninegar, *Facts About Sugar* 21, 639 (1926).

<sup>14</sup> J. deHaan, *Nederland. Tijdschr. Geneeskunde* 66, 1076 (1922).

<sup>15</sup> C. Kocour, U. S. Patent 1,806,806 (1931).

<sup>16</sup> Asamatsu Hyuga, U. S. Patent 1,659,529 (1928).

Direct readings of hydrogen-ion concentration can be made with an instrument wherein the sample with indicator is matched by the sample without indicator by interposing two suitable colored wedges which are either hollow and filled with solution and indicator, or are of solid colored glass.<sup>17</sup> By a complex arrangement of containers for solutions the wedge principle has been further applied to a rotating colorimeter.<sup>18</sup> A modified form of pH comparator, using sealed tubes, was developed for use in the Chicago Sanitary District. The tubes are of only 6 mm. outside diameter and only 1 cc. of sample is required. Results are obtained in one minute. By the use of such small tubes against a white background the effect of turbidity and natural color is minimized.

Rechecking of solutions, even in sealed tubes, is necessary at intervals, as all color standards will eventually change due to solution of glass, fading by exposure to light, and other factors.

**Spot Plate**—A less accurate form of series of standards apparatus uses 2 spot plates, bottles of indicator solution, pipets and color tables.<sup>19</sup> It is particularly advantageous for pH work with viscous liquids.

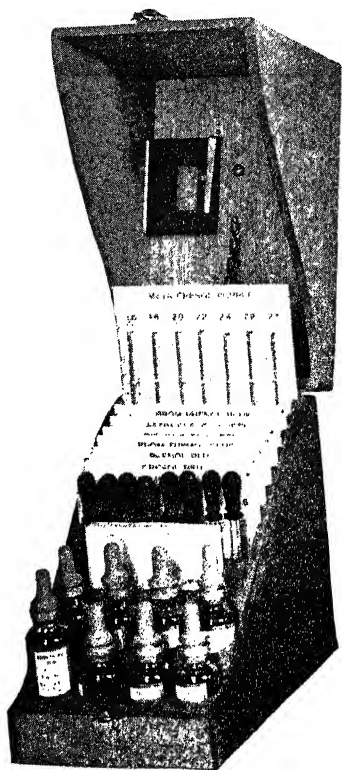


FIG. 10

S. D. C. Colorimeter for pH Determination. (Courtesy of Rascher & Betzold, Inc.)

<sup>17</sup> Eugene D. Stirren, U. S. Patent 1,616,092 (1927).

<sup>18</sup> Rene Andre Legendre, British Patent 285,848 (1927).

<sup>19</sup> G. Gollnow, *Riet en Biet* 6, 159-61 (1931); *Chem.-Ztg.* 57, 374-5 (1933).

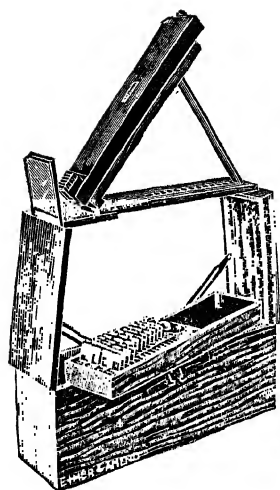


FIG. 11

Lovibond Tintometer  
(Courtesy of Eimer & Amend)

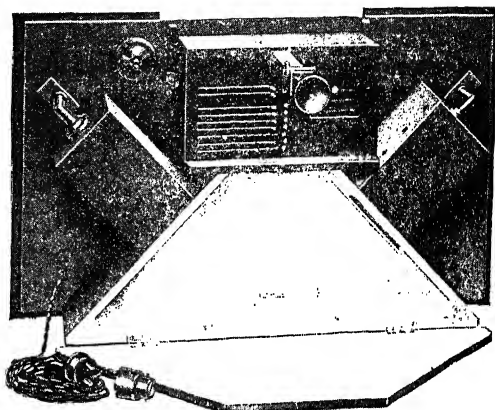


FIG. 13

Sample in Horizontal Lovibond Tintometer Using  
Artificial Light

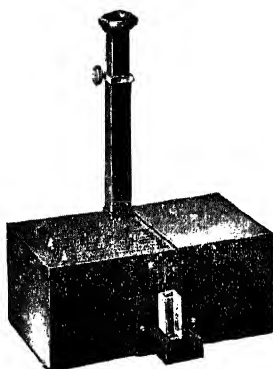


FIG. 12

Simple Form of Lovibond Tintometer Lovibond Tintometer for pH Determination  
(Courtesy of The Tintometer, Ltd.)



FIG. 14



## CHAPTER III

### APPARATUS—COMPARISON WITH SOLID STANDARDS

**Lovibond Tintometer**—One of the best known methods of estimating the color of a solution in terms of glasses is by the Lovibond tintometer. This has been calibrated for many quantitative determinations as well as for reading the colors of numerous liquids for comparison.

The apparatus consists essentially of a cell or tube to contain the sample, the color of which is compared with standard glasses, colored red, blue and yellow. In one form of instrument <sup>2</sup> a cell is used having

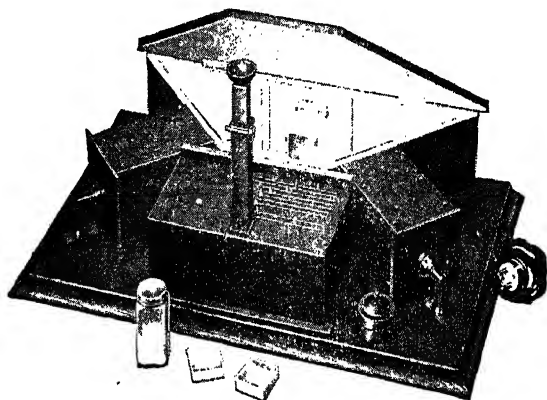


FIG. 15

Vertical Form of Lovibond Tintometer for Use With Artificial Light  
(Courtesy of The Tintometer, Ltd.)

parallel sides and a definite thickness of solution is read in terms of color glasses. The cells used vary from 1/16 to 18 inches in thickness.

In another form a wedge-shaped cell of sample is used <sup>3</sup> so that the thickness of sample can be varied over a wide range. In another form a tube <sup>4</sup> is used to provide for variable depths of solution as indicated by calibrations on the tube. This form requires that the horizontal or

<sup>2</sup> Joseph W. Lovibond, British Patent 12,867 (1886).

<sup>3</sup> Joseph W. Lovibond, British Patent 3859 (1887).

<sup>4</sup> Joseph W. Lovibond, British Patent 14,926 (1910).

inclined instrument be altered to a vertical form. Change of color in therapeutic pastilles as a result of exposure to ultraviolet radiation is measured by another type.<sup>5</sup>

Two methods of observation of colors are used with the Lovibond tintometer. Ordinarily no optical system is included and the eye compares two parallel colors. For the other method a set of prisms and an eyepiece is provided, similar to those of the Duboseq colorimeter.<sup>6</sup>

The Rosenheim-Schuster colorimeter<sup>7,8</sup> is designed to supply color glasses mechanically, in position for comparison with a sample in a cell or tube of fixed thickness. The glasses are in horizontal carriers controlled by colored knobs in front of the apparatus. Seven scales are ordinarily used. Six of these are red, blue and yellow from 1 to 10 and from 0.1 to 1.0, Lovibond units. The seventh carries extra, highly-colored standards. Provision is made for insertion of neutral grey slides if the sample is too brilliant in color for easy comparison. The instrument was originally designed for work with vitamin A. Such determinations, according to the English method, are made within 30 seconds for the arsenic chloride method, and within 1 minute for the antimony chloride method. The blue color produced is modified by the yellow and red of the oil. The apparatus is applicable to other determinations.

The usual forms of the Lovibond tintometer reflect light from a milk glass plate or layer of powder. Another instrument<sup>9</sup> provides for artificial light of 100 watts reflected from a standard white powder. This is held in place by a slightly bluish glass of a value not less than 0.5 or more than 1.95 on the Lovibond scale and not more than 0.15 inch thick. This glass is such as to correct for the excess of red and yellow rays emitted by the lamp.

By the use of a suitable reflector, color of a stationary object or of a furnace is estimated.<sup>10</sup> The principle is thus applied to pyrometry.<sup>11</sup>

The instrument has been used for recording and comparing colorimetrically a multitude of colored materials. The standard glasses come in sets for dyes, for fabrics and solids, for malt, sugar, wine, caramel and spirit, for ammonia by the Nessler method, for carbon in steel, for oils,

---

<sup>5</sup> Tintometer, Ltd., British Patent 277,166 (1927).

<sup>6</sup> See p. 43.

<sup>7</sup> Otto Rosenheim, *Biochem. J.* 21, 1329-34 (1927).

<sup>8</sup> Otto Rosenheim, E. H. J. Schuster and Tintometer, Ltd., British Patent 299,194 (1927).

<sup>9</sup> Tintometer, Ltd., British Patent 263,924 (1925).

<sup>10</sup> Joseph W. Lovibond, U. S. Patent 987,148 (1911).

<sup>11</sup> Joseph W. Lovibond, *Pottery Gaz.* 35, 1269 (1910).

waxes, lards, fats, gelatine, varnish, etc., for cottonseed oil, for petroleum oil, for flour, for tannin and for general purposes.

For the vegetable oil industry<sup>12</sup> an instrument having 4 circular discs permits the insertion of 4 glasses at a time in the field. The colors used are 0.1 to 0.9 red, 1.0 to 10.0 red, 1 to 9 yellow and 10, 20, 30 and 35 yellow. The 35 yellow is the basis for refined cottonseed oils. The color produced by combining 2 color glasses, while not the same as of the single glass, is so nearly so as to be within the necessary accuracy. A standard light and color filter are used to give standard readings.

The recommended standard set for vegetable oils contains 50 yellow glasses and 70 red glasses.<sup>13</sup> They are used with the enclosed Wesson type of colorimeter, a modified and simplified Lovibond, for corn and soybean oils. Modified forms permit greater ease of manipulation.<sup>14</sup> While a type of colorimeter reading in terms of spectral transmission curves would be more satisfactory, cost considerations dictate the continued use of Lovibond standards for routine work.<sup>15</sup>

A method for mixing and automatically delivering the mixed liquids to the colorimeter for examination has been patented,<sup>16</sup> the method of examination being subject to choice. For matching colored cloths with the instrument, uniform illumination is necessary.<sup>17</sup> A comparison with other methods has been published.<sup>18</sup>

**Instruments Similar to the Lovibond Tintometer**—Prior to the first patent by Lovibond an instrument for comparison of a standard oil with an unknown oil was patented.<sup>19</sup> In that, it was provided that a colored disc might be substituted for the standard oil.

An instrument similar to the Lovibond uses plates prepared with Prussian blue and lead chromate or picric acid.<sup>20</sup> Another instrument reads the color of petroleum oils in terms of color glasses, using artificial light.<sup>21</sup> Another patent<sup>22</sup> provides several wheels carrying colored

<sup>12</sup> H. S. Bailey, *J. Oil and Fat Ind.* 2, 8-13 (1925).

<sup>13</sup> W. D. Hutchins, *Oil and Fat Ind.* 8, 303-4 (1931).

<sup>14</sup> W. D. Hutchins, *Oil and Soap* 7, 135 (1934).

<sup>15</sup> L. M. Gill, *Oil and Soap* 7, 123-4 (1934).

<sup>16</sup> Ralph L. Rees, British Patent 291,174 (1928).

<sup>17</sup> E. Schell, *J. Soc. Leather Trades Chem.* 2, 297-9 (1918).

<sup>18</sup> L. P. McHatton, *J. Oil Colour Chemists Assoc.* 4, 189-204 (1921).

<sup>19</sup> Robert Porter Wilson, British Patent 3027 (1870).

<sup>20</sup> Klas Söndén, *Arkiv Kemi, Mineral. Geol.* 8, No. 7, 10pp (1921).

<sup>21</sup> Thomas D. Simpson and A. Slayton Woodman, U. S. Patent 1,495,763 (1924).

<sup>22</sup> J. P. Bader, U. S. Patent 1,629,609 (1927).

glasses for comparison. It is particularly suitable for petroleum oils. A modified form is used for measurement of the color of flowers.<sup>23</sup> The composite shade of a mixture of colored threads can be read.<sup>24</sup> A special instrument is used for the phosphorus determination by the molybdenum blue method in which the depth of sample is varied to match a blue glass standard.<sup>25</sup>

In another instrument to measure temperature by color change,<sup>26</sup> a gelatine filter absorbs all colors except red and green. A second, wedge-shaped filter absorbs red according to the thickness interposed, but no green. A third wedge-shaped filter absorbs green. The temperature of the light source is thus measured over the range of 900-2000° with an error of  $\pm 13^\circ$ . The Duboscq instrument has also been modified by replacing the standard by a colored glass.<sup>27</sup> One application is in common use for work on blood.<sup>28</sup>

Production of the various possible gradations of the primary colors has been provided for in an instrument having three graded color discs.<sup>29</sup> For furnishing a graded series of colors, such as are given by hydrogen-ion indicators, two suitably colored glass wedges are used.<sup>32</sup> This corresponds to the block comparator method but avoids the necessity of using tubes of liquids.

Color of turpentine may be read in terms of color glasses.<sup>33</sup> At the end of an eyepiece are two prisms suspended in a cell of turpentine. With the prisms at the center, artificial lights at the two ends are adjusted until the light from both ends is the same. A No. 1 Lovibond glass is then placed at one end and a No. 2 glass at the other and the prisms moved until the colors again match. Twice the depth that the eyepiece has been moved is the depth of turpentine necessary to match a No. 1 glass. Readings to 2 per cent are obtained. The principle should be applicable to other determinations.

---

<sup>23</sup> Herbert F. Roberts, *Plant World* 22, 262-9 (1919).

<sup>24</sup> P. Bourcart, *Chem.-Ztg.* 35, 577 (1912).

<sup>25</sup> Luigi Losana, *Giorn. chim. ind. applicata* 4, 60-2 (1922).

<sup>26</sup> G. Naeser, *Stahl u. Eisen* 49, 464-6 (1929).

<sup>27</sup> W. L. Patterson, U. S. Patent 1,643,515 (1927).

<sup>28</sup> H. S. Newcomer, *J. Biol. Chem.* 55, 569-74 (1923).

<sup>29</sup> Ferdinand V. Kallab, German Patent 193,814 (1905); U. S. Patent 862,336 (1907); German Patent 205,271 (1908).

<sup>32</sup> M. S. Badollet, J. Hamilton and C. F. Walton, Jr., U. S. Patent 1,505,185 (1924).

<sup>33</sup> C. F. Sammett, *J. Ind. Eng. Chem.* 8, 519-21 (1916).

**Hellige Colorimeter and Related Types**—This instrument<sup>34</sup> compares the sample in a square or rectangular cell with a series of colored glasses mounted in a rotating disc. It is designed for determination of hydrogen-ion but has been expanded by providing suitable color discs for ammonia, chlorine, nitric acid, nitrous acid, hydrogen sulfide, copper, iron, lead, manganese, titanium, nickel, phosphorus, oil, varnish, malt and beer, hemoglobin, creatinine, blood, bromides, sugar, uric acid, cholesterol and bilirubin determinations.

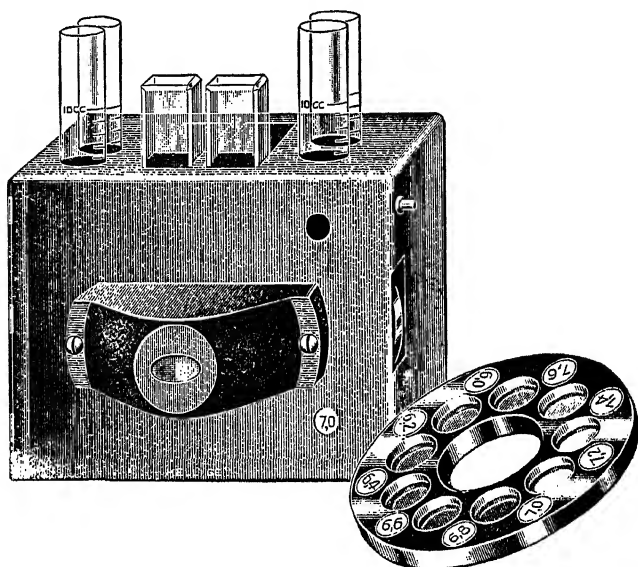


FIG. 16

Hellige Laboratory Comparator. (Courtesy of Hellige, Inc.)

A schematic diagram of the instrument is shown in Figure 17. For ordinary use A contains water or untreated sample and B the test solution with color developed. The water in A serves to make the path of liquid, through which the light must travel, the same in each case. The color disc CD is revolved to bring various glass standards into position at S so that one superimposed on A matches the color of B. Tubes C and D provide for the Walpole technique with a colored or turbid solution without the color disc. In that case Tube A contains a standard, C contains the untreated sample, B contains the treated sample

<sup>34</sup> Paul A. E. Hellige, U. S. Patent 1,870,624 (1932).

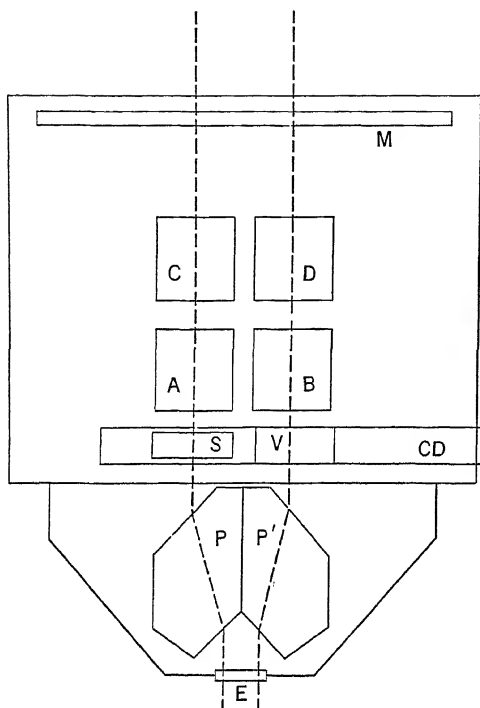


FIG. 17

Schematic Diagram of Hellige Comparator

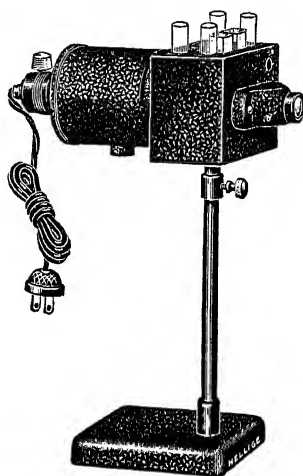


FIG. 18

Hellige Comparator With Illuminator

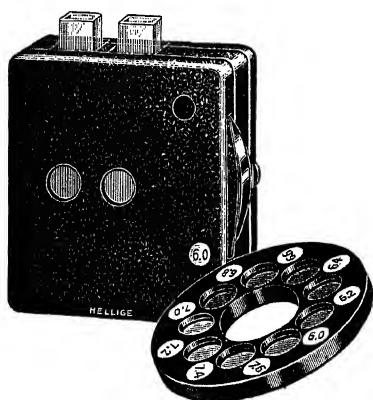


FIG. 19

Simple Hellige Comparator

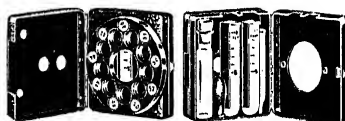


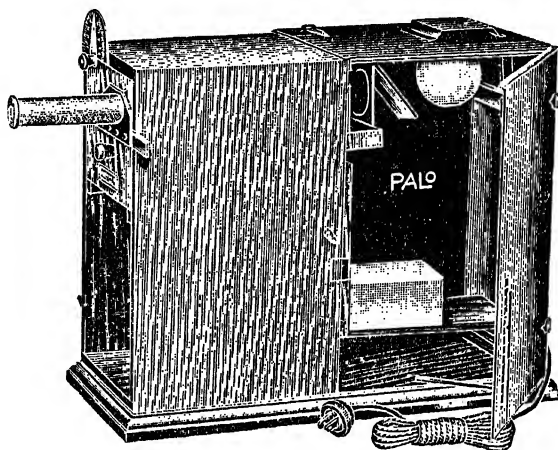
FIG. 20

Hellige Pocket Comparator

(Courtesy of Hellige, Inc.)

and D contains water. Tubes C and D may also be used to superimpose filter colors on A and B. M is a ground glass available in either white or blue.

The dotted lines show the path of the light rays. Passing through A and B with or without C and D they are refracted by the Helmholtz double plate, P,P', and joined in an eyepiece at E. The color disc is mounted with a large hollow center V through which the light passes from B. Both pocket and laboratory types are available. Very satis-



. 21

Ives Tint Photometer. (Courtesy of Palo-Myers, Inc.)

factory results have been reported.<sup>35</sup> A micro instrument uses a capillary tube of sample. A similar type of instrument is offered by British Drug Houses, Ltd. The Wulff type uses colored strips with indicators<sup>36</sup> for comparison of pH values. Its main field is for quick plant tests.

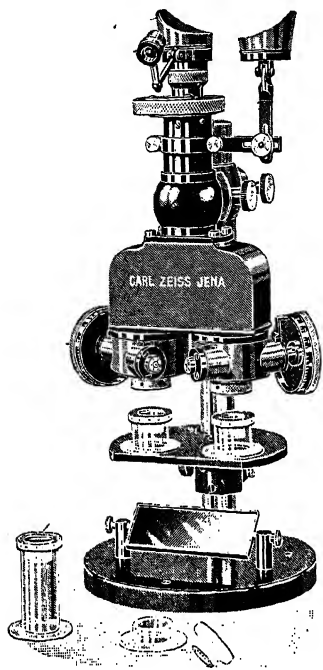
**Tint Photometers**—Probably the best known form of tint photometer is that of Ives<sup>37</sup> in which the color from the specimen is compared with that of a magnesia block reflected through color glasses and a variable aperture. The three color components selected are red, blue and green. Other colored slides are also available. By omission of the color glasses

<sup>35</sup> B. Drews, *Z. Spiritusind.* 52, 359-60 (1920).

<sup>36</sup> H. Janistyn, *Seifensieder-Zig.* 58, 776-7, 791-2 (1931).

<sup>37</sup> Frederic E. Ives, *J. Franklin Inst.* 164, 47-56, 421 (1907); U. S. Patent 1,457,076 (1923).

the instrument is used as a photometer for measuring greys. The color glasses of the Ives tintometer are relatively accurate but variations have been reported.<sup>39</sup> Fundamentally this instrument is not a colorimeter, in the sense of being an instrument for colorimetric estimation of metals or radicals. A similar principle in elementary form is involved in an instrument using colored celluloid standards for reading colors of opaque and transparent solids and liquids.<sup>40</sup>



Pulfrich Photometer. (Courtesy of  
Carl Zeiss, Inc.)

A typical use of the Ives instrument is for measuring the decolorizing effect of vegetable carbon on a sugar solution.<sup>41</sup> In the authors' laboratory this instrument has been found very useful for colorimetric hydrogen-ion work, the color in standard tubes of sample and standard solutions being read with the instrument and compared numerically. Since light greys may be more accurately compared than darker colors, the colors of the sample and standard may be combined with the complementary color and the light greys so produced compared.<sup>42,43</sup> The color of the light admitted to the pair of tubes may be varied.

A modified form of tintometer reconstitutes a given color without the use of a rotating device.<sup>44</sup> A rectangular beam of light of constant cross section passes through 2 glass slides. On each slide 4 or more color screens are mounted. Each slide covers half of the beam of light.

Each screen covers the whole or a measured fraction of the beam. The beam is then condensed with a convergent lens and directed through a small opening into a sphere. The inside of the sphere is painted a dull white and is observed through a small opening at right angles to the enter-

<sup>39</sup> E. C. Bryant, *Astrophys. J.* 55, 9 (1922).

<sup>40</sup> Ralph L. v. Kelmperer and F. Lowe, *Chem.-Ztg.* 36, 853-5 (1912).

<sup>41</sup> F. W. Zerban, *Louisiana Planter* 61, 282-3 (1918).

<sup>42</sup> Melvin Moody, U. S. Patent 1,389,836 (1921).

<sup>43</sup> A. Thiel, *Z. anal. Chem.* 94, 170-7 (1933).

<sup>44</sup> G. Bernheim and A. Guntz, *Chimie & industrie*, Special No. 175 (March 1932).



ing beam. By application of the apparatus the tone of a shade is measured by the proportions of the fundamental colors required to produce it. The intensity of the shade is measured by the size of aperture.

**Colorimetry without Standards**—The use of a colorimeter without a standard refers to an absolute method of measurement in which a calibration curve or table is used. The Pulfrich photometer, as shown in Figure 22, is typical of equipment for this purpose. A similar, but less expensive, instrument is mounted horizontally.

Two parallel telescopes with the usual system of prisms join the images as halves of a circle.<sup>45</sup> At the rear of the eyepiece is an opening for insertion of color filters in the path of the rays. The measurements are made by micrometers mounted at the openings of the telescopes. By movement of these the intensity of the field of view is varied in a manner which can be measured numerically. The accuracy for faintly and deeply colored liquids is approximately identical by varying the depth of the colored solution.

In one of the tubes on the stage place the solution to be examined. The other contains the same solution in which color has not been developed. By adjustment to equal illumination through a suitable color filter, an absolute value is obtained, measuring the color developed in the sample. The instrument, by suitable modification, may be used to vary the depth of sample or standard in the same way as the usual types of balancing instruments. It has a modified nephelometric form which may also be used in measurement of the intensity of fog or smoke.

**Trichromatic Colorimetry**<sup>46,47,48</sup>—This type of instrument depends on the mixing of light from three selected standards. It may be contrasted with the Lovibond method as being additive rather than subtractive colorimetry.<sup>48a</sup> In it colors are mixed to give the desired sensation rather than subtracting the necessary wave lengths from white light to give the sensation. No standards can be selected which will match all possible colors. By addition of a determined amount of one primary or of white light to any sample color, it can be matched by a suitable set of

<sup>45</sup> See p. 44.

<sup>46</sup> J. Guild, *Trans. Opt. Soc.* 27, 106-29 (1925-6); *J. Sci. Instruments* 11, 69-78 (1934).

<sup>47</sup> I. Perry, *J. Soc. Dyers Colorists* 43, 159-61 (1927).

<sup>48</sup> J. W. Forrest, *Paper Trade J.* 97, 38-9 (1933).

<sup>48a</sup> G. S. Fawcett, *J. Soc. Dyers Colorists* 51, 90-3 (1935).

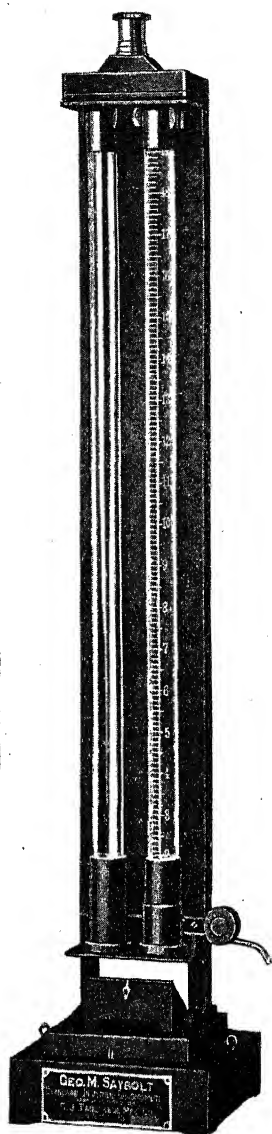


Fig. 23

Saybolt Universal Chromometer. (Courtesy of C. J. Tagliabue Mfg. Co.)

three standard colors. The instrument should mix three primary colors on the comparison side of the field and allow addition of a modifying color to the sample side when it cannot be matched directly. While saturated colors are not essential, practical limitations dictate their use. Suitable standards are Wrattan No. 71 (red  $0.63\mu$ ), Wrattan No. 49B (blue  $0.45\mu$ ), and Wrattan No. 62 (green about  $0.537\mu$ ). The latter is not ideal but is usable.

In practice three gelatine filters are used. The amount of each primary color admitted is gauged by an opening adjustable to a variable amount. By a rotating periscopic prism the three beams are combined and compared with a beam from the illuminated sample. Artificial illumination, corrected with a filter, is used for the sample. Irregularities due to texture of the sample are removed by a lens. If the sample reflects or transmits only a small amount of light a neutral screen is used with the standard. When the color of the sample is modified by addition of a white light or a spectral color, that is separately measured and deducted from the total reading.

The true color analyzer<sup>40</sup> is not applied to strictly colorimetric analysis.

**Petroleum Colorimeters**—An instrument has been evolved by combination of the Lovibond tintometer and by the Stammer colorimeter, known as the Saybolt chromometer. This instrument is intended solely for the evaluation of the colors of refined petroleum oils. This is similar to the Stammer instrument in that two tubes are provided, but the left-hand tube, A, is

<sup>40</sup> Carl W. Keuffel, U. S. Patent 1,524,180 (1925).

modified to an empty tube in which the color is obtained by the addition of 1 or 2 glass discs. The sample in B is drawn off until an amount is left which will duplicate the color of the disc or discs used. From the graduations the oil may then be graded in terms of the standards for

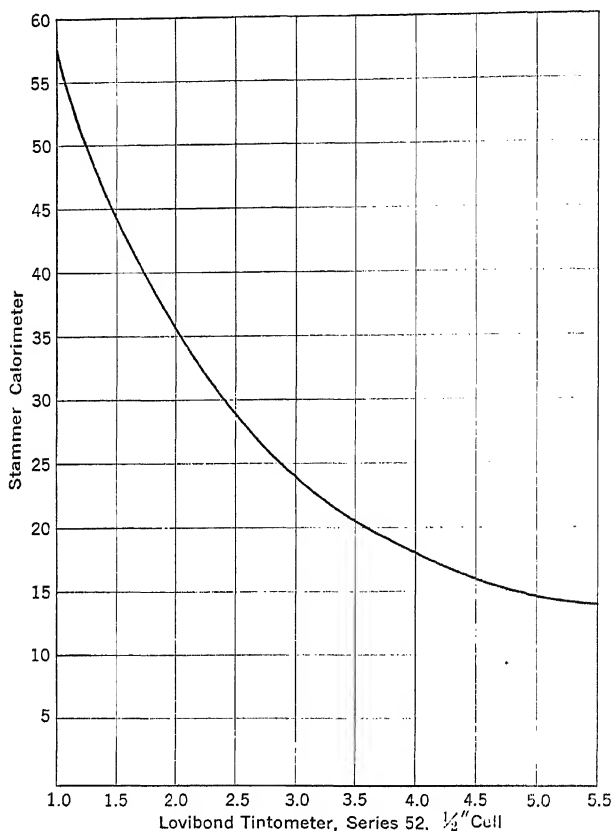


FIG. 24

Conversion of Lovibond to Stammer Readings

petroleum oils. Lovibond readings are converted into Stammer readings by a curve reproduced as Figure 24. This was developed with 8 per cent malt wort.<sup>50</sup>

The Tag-Robinson colorimeter for reading the colors of lubricating oils operates by raising and lowering the tube containing the sample, to vary the depth of color observed through a glass plunger. It is thus a modified

<sup>50</sup> C. A. Nowak, *J. Ind. Eng. Chem.* 6, 323-4 (1914).

Duboseq colorimeter. Comparison is with 3 glass standards and is claimed to duplicate all oil colors.

In another type of oil-testing instrument, the Union colorimeter, a 4-ounce oil-sample bottle of the oil or a special glass jar of sample is compared with 15 slides of graded color. If the sample is too dark it is diluted. Artificial lighting is provided and the method is a standard of the American Society for Testing Materials.

The Bader colorimeter developed by the Texas Company uses a standard tube of oil for comparison with a series of glasses. Conversion tables permit translation of the results into Saybolt, Union or Lovibond units.<sup>51</sup> Various oil colorimeters are used for different grades of oil so that no one instrument can be recommended for all grades.

For the orthotolidine test in water, a colorimeter, which should be generally applicable, has a color glass at the bottom of one tube and has the inside of the tubes threaded at about 30 threads to the inch to prevent reflection of light from the side walls.<sup>52</sup> This also flattens the meniscus. The liquid is drawn off as from Hehner cylinders. A bridge for insertion of glass standards in the Duboseq instrument is

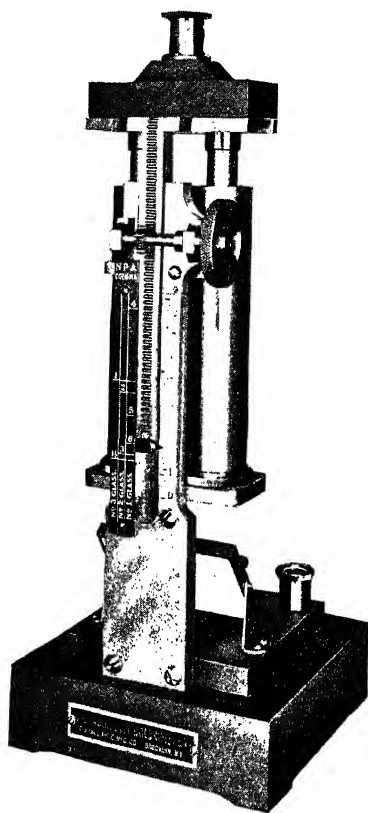


Fig. 25

Tag-Robinson Colorimeter. (Courtesy C. J. Tagliabue Mfg. Co.)

also available.<sup>53</sup> Its use is shown in Figure 27.

Practically all paraffin-base oils have essentially the same light absorption curve. Therefore, by using dilution or a thin film, they may also

<sup>51</sup> P. D. Barton, *Natl. Petroleum News* 14, 53-4 (1922).

<sup>52</sup> John C. Baker and Charles F. Wallace, U. S. Patent 1,794,134 (1931).

<sup>53</sup> Hellige, Inc., New York, N. Y.

be compared with a liquid standard by the Duboseq colorimeter instead of using color glasses.<sup>54</sup>

### Comparison with Color Plates—Comparison of colors with charts

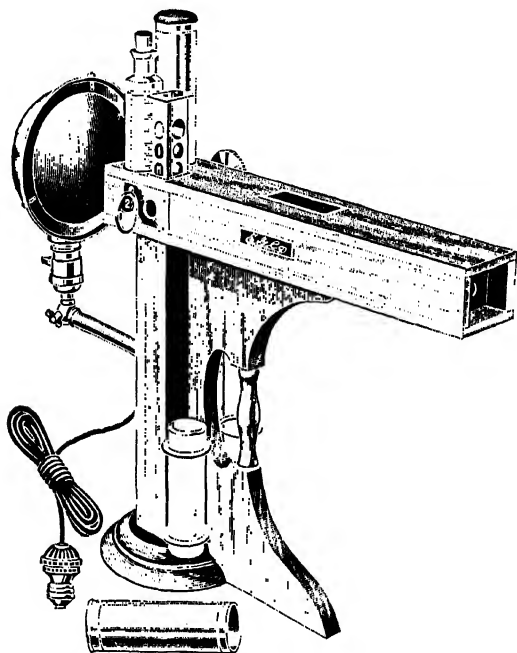


FIG. 26

Union Colorimeter. (Courtesy of Emil Greiner Co.)

is difficult at best. Clark has applied the method successfully to pH determinations.<sup>55</sup> Such a method has been suggested for the U. S. Pharmacopeia.<sup>56</sup> The American Oil Chemists Society has had poor results in attempts to compare colors of soiled fabric with either color plates or standard soiled fabrics.<sup>57</sup>

<sup>54</sup> L. W. Parsons and R. E. Wilson, *J. Ind. Eng. Chem.* 14, 269-78 (1922); *Ibid* 19, 74-8 (1927).

<sup>55</sup> W. Mansfield Clark, "The Determination of Hydrogen Ions," 3rd edition. Williams and Wilkins Co., Baltimore, Md. (1928).

<sup>56</sup> E. N. Gathercoal, *J. Am. Pharm. Assoc.* 12, 676-9 (1923).

<sup>57</sup> L. F. Hoyt, *J. Oil and Fat Ind.* 4, 29-34, 66-9 (1927).

**Color Glasses**—One important consideration in using these types of equipment is the accuracy with which the glasses can be duplicated. Considerable variations in standard color glasses have been reported.<sup>58,59</sup> The National Bureau of Standards found it necessary to recalibrate its standard set in spectrophotometric terms to remove inconsistencies.<sup>59a</sup> The glasses for the Lovibond tintometer vary in color from 0.1 to over 100

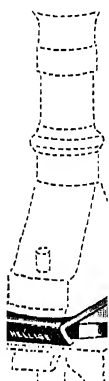


FIG. 27

Glass Standard in Place on Duboseq Colorimeter. (Courtesy of Hellige, Inc.)



FIG. 28

Glass Standards for Use With Duboseq Colorimeter. (Hellige, Inc.)

units of each, red, yellow and blue. The complete set of glasses comprises 470 units but no one type of work requires the complete set.

A calibration of 16 color glasses rated at 7.6 red was made at the Bureau of Standards.<sup>59</sup> The average was 0.1 unit lighter than the nominal value. One was 0.54 unit darker, the others all varied less than 0.33 unit from the rated figure. A study of the precision and reliability

<sup>58</sup> K. Zert, *Z. Zuckerind. Czechoslov. Rep.* 52, 57-63 (1927).

<sup>59</sup> I. G. Priest, *Cotton Oil Press* 4, 43-4 (1921).

<sup>59a</sup> Kasson S. Gibson and Geraldine Walker Haupt, *Oil and Soap* 11, 246-250, 257 (1934).

of grading at about 35.0 yellow, 7.6 red was made. The average deviation of a single observation from the mean was about 0.1 unit; the maximum deviation as much as 0.4 unit. Means obtained in the forenoon and afternoon differ from the general mean by 0.1 unit in some cases. The supposed accuracy of grading to 0.1 unit red on the color scale is not attained. The density and serial numbers of a complete series of glasses is almost linear but when extended, cuts the axis of 0 density at  $-2$ .<sup>61</sup> This is because filters of equal density at the wave length of maximum absorption of light are not equal when examined with white light.

For Lovibond glasses between 35 yellow and 0 red and 35 yellow 7.2 red on the Priest-Gibson scale an increase in temperature of  $25^{\circ}$  C. is practically equivalent to addition of 0.2 in Lovibond red units.<sup>62</sup> The temperature effect in grading of cottonseed oil is almost negligible. More recent work<sup>63</sup> has shown on 65 glasses that the discrepancy from the Bureau of Standards set is not more than 2-3 times the least difference perceptible with certainty, by the best observers under the most favorable conditions of observation. The variation from the average of the 65 glasses was much less. These irregularities were found by a method much more sensitive than those contemplated by the makers. Only 3 glasses badly marred were unsuitable for use under the present conditions of grading.

Regradings on 1000 red color glasses have been reported.<sup>64,65</sup> In general the red glasses supplied give readings higher than that for which they are marked. Such glasses, when recalibrated for the vegetable oil industry by the Bureau of Standards for use with 35 yellow, have an accuracy to 0.1 unit up to 10.0 and lesser accuracy above that point.<sup>66</sup> The yellow glasses are not particularly important as to lack of uniformity.

**Reported Variations**—The color of potassium chromate solution in a 2-inch cell is less than double that in a 1-inch cell when read in terms of color glasses.<sup>67</sup> It is well known that the sum of a series of fractional glasses is not a match for the glass to which they are supposedly equal. This is because transmission of light through a greater number of glass surfaces introduces a grey tint. The usual practice is to avoid the use of

<sup>61</sup> T. M. Lowry and L. P. McHatton, *J. Oil Colour Chem. Assoc.* 5, 351-3 (1922).

<sup>62</sup> Deane B. Judd, *Bur. Stan. J. Res.* 1, 866 (1928).

<sup>63</sup> Irwin G. Priest, Deane B. Judd, K. S. Gibson and Geraldine K. Walker, *Bur. Stan. J. Res.* 2, 793-819 (1929).

<sup>64</sup> Irwin G. Priest, *Oil and Fat Ind.* 6, 27-9 (1929).

<sup>65</sup> Geraldine K. Walker, *Bur. of Stan. J. of Res.* 12, 269-82 (1934).

<sup>66</sup> *Bureau of Standards Research Paper* 58, 819.

<sup>67</sup> F. W. Richardson, W. Mann and N. Hanson, *J. Soc. Chem. Ind.* 22, 614-6 (1903).

more than 4 slides when possible. An alternative, applied with partial success, is the addition of a neutral grey slide on the sample side of the apparatus. Discrepancies in the color readings of malt worts and beer were found to be due to insufficient light and remedied by artificial illumination.<sup>68</sup>

The Lovibond instrument has been reported as unsatisfactory<sup>69</sup> for determining the color of tanning materials and an instrument substituted in which the depth of tannin solution is varied to match a definite standard color. The same principle could be applied with the tube type of Lovibond instrument.

Comprehensive discussions of the Lovibond Color System have been published<sup>70,71</sup> and indicate both a need for calibration of Lovibond glasses and the difficulties in the way of such calibration. An incorrect glass may be in error in any or all of the three variables, hue, saturation and brilliance. The interference of reflection from multiple glasses is certainly important. Regardless of the errors of such solid standards, they are widely used for commercial standardization and reporting of colors, even though more satisfactory methods are available in many cases.

The honors in a recent discussion of liquid vs. solid standards<sup>72,73</sup> seem to the observer to rest with the liquid standards.

<sup>68</sup> Joseph W. Lovibond, *J. Inst. Brewing* 14, 2 (1908).

<sup>69</sup> H. R. Proctor, *J. Am. Leather Chem. Assoc.* 5, 352-60 (1910).

<sup>70</sup> K. S. Gibson and F. K. Harris, *Scientific Paper No. 547*, Bureau of Stan. (1927).

<sup>71</sup> F. E. Lovibond, *Proc. Optical Convention I*, 211-4 (1926).

<sup>72</sup> Arthur Schroder, *Ind. Eng. Chem.* 21, 481 (1929).

<sup>73</sup> W. A. Taylor, *Ind. Eng. Chem., News Ed.* 7, 7 (1929).



## CHAPTER IV

### APPARATUS—DILUTION AND DUPLICATION METHODS

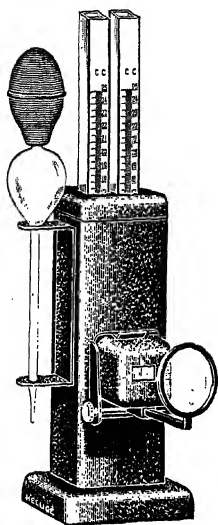
**Dilution Method**—The apparatus for comparisons by the dilution method consists essentially of a pair of graduated tubes. A convenient device for use with this method is a light-proof box, painted black inside, with holders for two tubes near one end. The end near the tubes is fitted with a ground glass screen. The other end is fitted to the face of the observer so that no side light may enter. By the use of this a more accurate judgment as to the colors of the two tubes may be made than if they are compared in the open. This apparatus does not tire the eyes of the operator as quickly as some of the colorimeters used for the balancing method. More modern equipment, but less restful to use, is shown in Figure 29. Such equipment brings the images of the 2 tubes into juxtaposition and is relatively inexpensive. In all cases possible, a colorimeter should allow the use of both eyes for making the comparison so as to lessen fatigue. Comparison of the two tubes may be made by holding them in the hand against a sheet of white paper.

Whether or not the camera is used for the comparison, the operation of the dilution method is the same. Beer's law is assumed to hold. Place standard and sample in two graduated tubes. The colors of the two solutions should be nearly alike. The experienced operator soon learns to choose his sample so that the resultant color will be nearly the same as that of the standard which he is using. Add water to the darker, carefully mixing after each addition, until the colors of the two solutions, observed horizontally through the tubes, appear to be identical.

When this point is reached the concentration of the test substance in each solution must be the same and their contents are then related to each other as the volumes. Since the amount of test substance in the amount of standard used is definitely known, it is easy to calculate the test substance in the sample by a direct proportion. Care should be taken that the tubes used are clear and free from flaws and that the thickness of glass, internal diameter and graduations are identical. Matched pairs of Nessler tubes are sold for this purpose.

The curved Eggertz tube for carbon determinations is applicable to

the dilution method. This has a bend about 2 inches from the top so that the tube may be lightly shaken to mix the water added without danger of the contents being spilled. A special dilution type of instrument has been described<sup>1</sup> and later modified by addition of an artificial source of light.<sup>2</sup> It is rather involved, as a device is provided whereby the eye does not have to be removed from the eyepiece. A cord is pulled



g. 29

Dilution Colorimeters. (Courtesy of Hellige, Inc.)

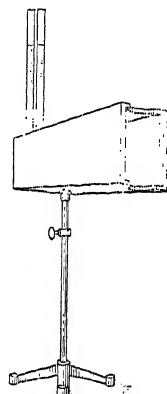
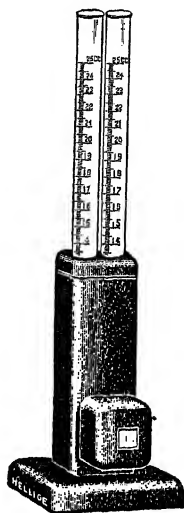


FIG. 30

Color Camera

to allow liquid to drop slowly from a buret into the standard tube. Mechanical stirring is also provided.

The dilution method is probably the most suitable for class use.<sup>3</sup>

**Duplication Method**—Determinations by the method of duplication are usually carried out in graduated Nessler tubes. First dilute the sample to some convenient, definite volume. Then add the same reagents as were added to the sample to a volume of water amounting to nearly the volume of the sample. The amount of water used for the blank varies according

<sup>1</sup> R. V. Stanford, *Z. physiol. Chem.* 87, 159-71 (1914).

<sup>2</sup> R. V. Stanford, *Biochem. J.* 17, 839-43 (1923).

<sup>3</sup> E. Guy Hopkins, R. F. McCracken and W. F. Sharpe, *Bull. Med. Coll. Va.* 23, No. 3, 24-6 (1926).

to the concentration of the standard to be used. To the blank containing the same reagents as those used for the sample add a standard solution, carefully mixing after each addition, until the color of the sample is duplicated by that produced in the blank. The color of the blank having been made to duplicate that of the sample, they may still differ in volume. It is simple enough to calculate the amount of standard that would be necessary if the volumes were equal.

To eliminate error and to correct for the variation in salt concentration which would thus result between sample and standard, duplicate the volume as well as the color of the standard. This is accomplished by addition of water and standard alternately until the two solutions are identical in both color and volume. The same precautions apply to this method as to the dilution method so far as apparatus is concerned. The tubes used must be of the same size, thickness of glass and internal diameter and the graduations on the outside must correspond in height. Beer's law need not hold.

## CHAPTER V

### APPARATUS—BALANCING METHOD

THE apparatus for the balancing method is the most elaborate of all and its use the simplest. The various instruments used include Hehner cylinders, Nessler tubes and various types of colorimeters. The apparatus for this method may be subdivided into types in which the actual depth of standard liquid used is varied, and those in which it appears to be varied. The second type is further subdivided into cylinder types and wedge types.

**Nessler Tubes**—In its most elementary form the apparatus for the balancing method consists of two similar graduated cylinders. Place the sample in one tube and hold them over a reflecting surface such as a sheet of white paper. Pour standard solution into the standard tube until the color observed through the lengths of the cylinders is identical. The amount of standard used and its content of the test substance per unit volume are known. The test substance in the sample is then inversely proportional to the volume, if the content per unit volume is desired, or the total amount of test substance in the two solutions is identical. If the diameters of the two cylinders are not identical, the strengths of the solutions are inversely proportional to their depths. For accuracy the concentrations of sample and standard must be similar. A great number of devices have been developed for increasing the accuracy with which the color of the two tubes can be matched, and for simplifying the operation.

**Hehner Cylinders**—Hehner cylinders consist of two glass tubes with flat bottoms. Each has a side tube with stop-cock near the bottom. For a determination, place the solution of sample in one tube. Partially fill the other tube with the standard so that the depth of color seen by looking downward through the length of the column of liquid is deeper than that seen by similarly looking down through the sample. Draw off standard until the colors of the 2 tubes observed in this way are the same. Read

the depths of solution in the two tubes, or, if their diameters are identical, the reading may be taken by volume.<sup>1,1a</sup>

A case for the Hohner cylinders practically converts them into a balancing type of colorimeter.<sup>2</sup> Another colorimeter is based on the principle of two Hohner cylinders connected with rubber tubing to

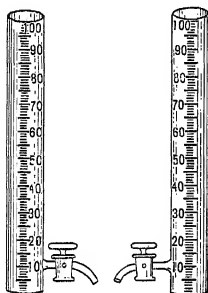


FIG. 31  
Hohner Cylinders

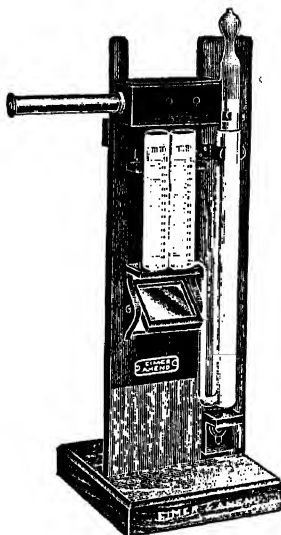


FIG. 32  
Campbell-Hurley Colorimeter.  
(Courtesy of Eimer & Amend)

movable reservoirs. The former are located over a mirror and compared by balancing.<sup>3</sup>

**Campbell-Hurley Colorimeter**—As the next stage of development, the column of liquid in one cylinder is fixed at a definite level and that in another varied by a mechanical method of addition or removal. Such an instrument may be relatively inexpensive. The Campbell-Hurley type of colorimeter illustrated is the common form of such an instrument.<sup>4</sup> This is a modification of the Kennicott-Sargent type of apparatus and is

<sup>1</sup> F. Toggenburg, *Schweiz. Wochschr.* 50, 417-9 (1912).

<sup>1a</sup> Cf. N. P. Komar, *J. Applied Chem. U. S. S. R.* 7, 420-3 (1934).

<sup>2</sup> T. Gunther, *Chem.-Ztg.* 33, 318 (1909).

<sup>3</sup> R. C. Frederick, *Analyst* 52, 469-70 (1927).

<sup>4</sup> E. D. Campbell and W. B. Hurley, *J. Am. Chem. Soc.* 33, 1112-5 (1911).

## APPARATUS

sometimes known as the Kennicott colorimeter. The unknown solution is placed in tube A and, since the volume can be readily governed so as to come to some even graduation, markings are only placed at five mm. intervals. The standard solution is placed in the right-hand tube B which is graduated to single mm. Tube B is permanently connected by a glass tube with the reservoir C in which the glass plunger D works. The level of the liquid in B may be readily controlled by raising or lowering the

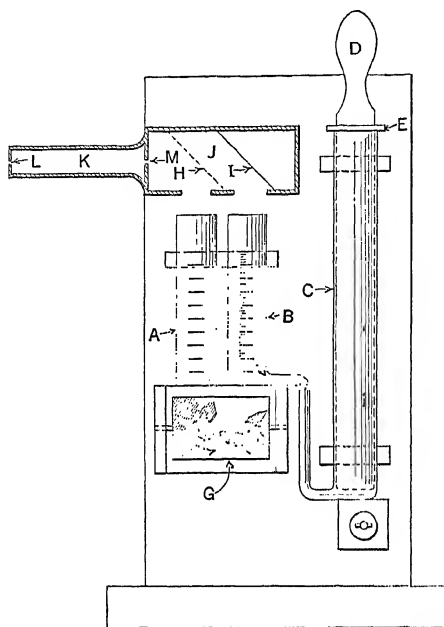


FIG. 33

Schematic Diagram of Campbell-Hurley Colorimeter

plunger. As the tube B and the reservoir C are made in one piece the standard comes in contact with glass only, thus preventing contamination by rubber or cork. The plunger D is provided with a rubber collar E to prevent it from coming into contact with the bottom of the reservoir. Tubes A and B and reservoir C rest on wooden supports with holes under A and B for the passage of light. All glass parts are held in place by spring clips which allow easy removal for cleaning.

For operation turn the colorimeter with the back toward a window, preferably a north one, and adjust the mirror G to reflect skylight upward

through A and B. By this arrangement the back of the colorimeter serves as a screen to cut off light, except that reflected from G. The light passing through tubes A and B impinges on the two mirrors H and I cemented to brass plates sliding in grooves cut at an angle of  $45^\circ$  in the sides of the wooden box J. This box has a loosely fitted cover so that it may be removed for cleaning the mirrors. The mirror H is cut vertically and cemented in such a position as to reflect one half of the circular field of light coming through tube A. The light, passing upward through B, is reflected horizontally by the mirror I through a hole in the brass plate supporting the mirror H. One-half of the circular field of light from the tube B is cut off by the mirror H, the vertical edge of which acts as a dividing line between the two halves of the circular field. The image of one-half of the tube B is then observed in juxtaposition to the opposite half of the image of the tube A.

The images are observed through tube K, 2.5 cm. in diameter and 16 cm. long, lined with black felt and provided with an eyepiece having a hole 1.5 mm. in diameter. At point M in tube K is placed a diaphragm having an aperture 8 mm. in diameter. All parts inside the box J except the mirrors are painted black so that no light except that coming through the tubes A and B passes through K. By having the apertures in the eyepiece and the diaphragm properly proportioned only the images of the bottoms of tubes A and B can be seen, preventing the interference of side light.

A person looking through the eyepiece observes a single circular field divided vertically by an almost imperceptible line when the two solutions are of the same intensity of color. By manipulating plunger D the level of the liquid in B can be raised or lowered, thus causing the right half to assume a darker or lighter shade at will. In matching colors with an ascending column in B, that is, gradually deepening the color of the right half of the field, the usual tendency is to stop a little below the true reading, while in comparison with a descending column the opposite is the case. The operator should take a reading in each direction until after a little practice this tendency to error has been overcome.

**Modified Campbell-Hurley Instruments**—For work in ether solutions a modified Campbell-Hurley instrument is used. The mechanical principles are the same.<sup>5</sup> The tops of the tubes are ground flat to take tightly fitting glass covers. Pressure is obtained by raising and lowering

---

<sup>5</sup> Walker J. King, *Ind. Eng. Chem.* 15, 350-2 (1923).

a water reservoir which transmits pressure through an air column to the ether solution.

For work in the absence of oxygen the principle has been carried further in an instrument having sealed plates on the tops of the tubes and an additional side arm or arms. The standard tube contains oxygen-free carbon dioxide which is liberated through a stopcock for admission of more standard. The sample is introduced into carbon dioxide through one side arm with provision for allowing the gas to escape through another, also located near the top of the cylinder.<sup>6</sup> The change of color of free radicals with dilution was investigated in this instrument. The same principle has been applied to comparison of Nessler tubes, the optical portion of the apparatus being the same.<sup>7</sup>

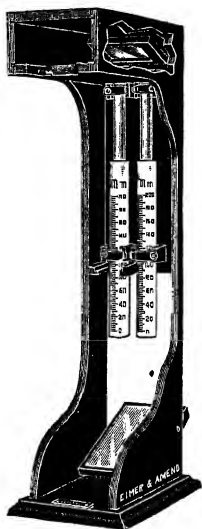


FIG. 34

Schreiner Colorimeter.  
(Courtesy of Eimer  
& Amend)

By insertion of an iris diaphragm with a suitable indicator for reading the degree to which it is closed, an instrument developed from a pair of Nessler tubes can be used for measurement of turbidity. In practice the Campbell-Hurley type of instrument was so modified.<sup>8</sup>

Reflection from a mirror in a trough of solution has also been used, the trough being movable with respect to the mirror. The principle is therefore like the Campbell-Hurley instrument.<sup>9</sup> This principle has been applied to examination of turpentine.

**Schreiner Colorimeter**—The Schreiner<sup>10</sup> colorimeter has two tubes similar to Nessler tubes but graduated in mm. of depth instead of by volume. These are held by brass clips and move up or down on two smaller hollow glass plungers.

The principle is intermediate between the simple use of Nessler tubes and the Duboseq colorimeter. The latter is much easier to use both mechanically and optically. This instrument was devised primarily

<sup>6</sup> M. Gomberg and F. W. Sullivan, Jr., *J. Am. Chem. Soc.* 44, 1810-35 (1922).

<sup>7</sup> John H. Yoe, *Ind. Eng. Chem.* 19, 1131 (1927).

<sup>8</sup> P. L. Hibbard, *Ind. Eng. Chem.* 16, 804-5 (1924).

<sup>9</sup> George Steiger, *J. Am. Chem. Soc.* 30, 215-9 (1908).

<sup>10</sup> O. Schreiner, *J. Am. Chem. Soc.* 27, 1192 (1905).



for examination of soil extracts. By a modification, the optical system joins the two images in the usual split circle.<sup>11</sup> Other modifications have been described.<sup>12</sup> Comparison may also be made with the color of standard glass slides using this instrument.

**Duboscq Colorimeter**—In the Duboscq type of instrument the same

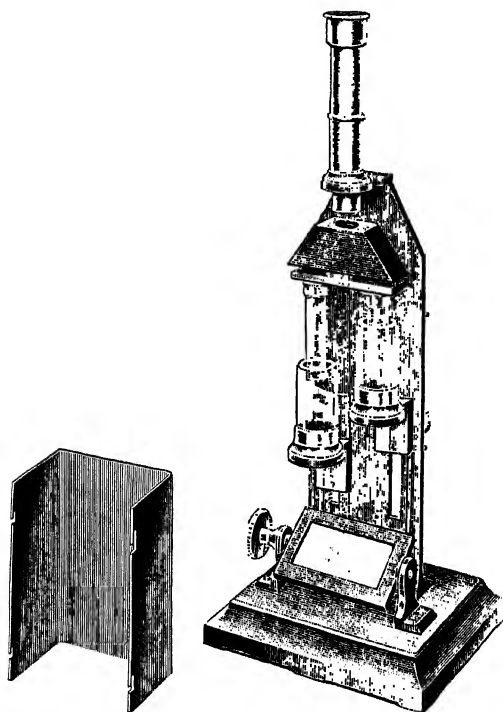


FIG. 35

Standard Duboscq Colorimeter

result as in the Campbell-Hurley type is obtained by a different method. The two independent tubes, A and B, are of the same size and hold the solutions of the unknown and the standard. Each is mounted in a holder, M, N, which slides up and down in a slit cut in the backboard of the instrument and is operated by a rack and pinion. Light is reflected

<sup>11</sup> C. W. Davis, *J. Franklin Inst.* 190, 243-4 (1920).

<sup>12</sup> N. Roberts, *Bull. Hyg. Lab. P. H. and M. H. Serv.* 66, 3 (1910).

upward through the tubes by a mirror G. Directly over tubes A and B which contain the solutions to be compared are two glass plungers, O, P, of a diameter less than that of A and B. The bottoms of these plungers are

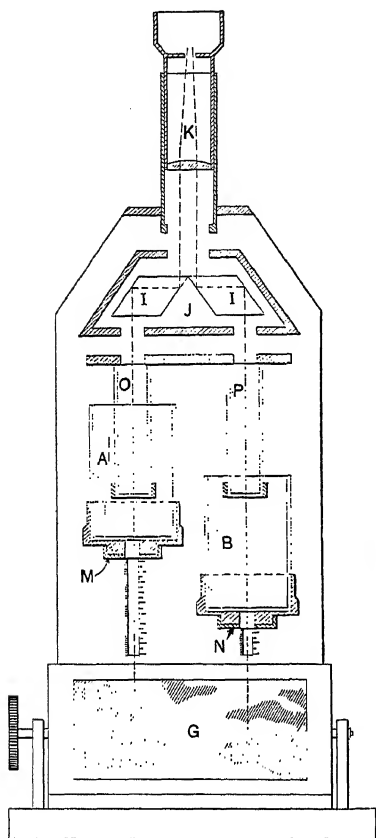


FIG. 36

Schematic Diagram of Duboseq Colorimeter

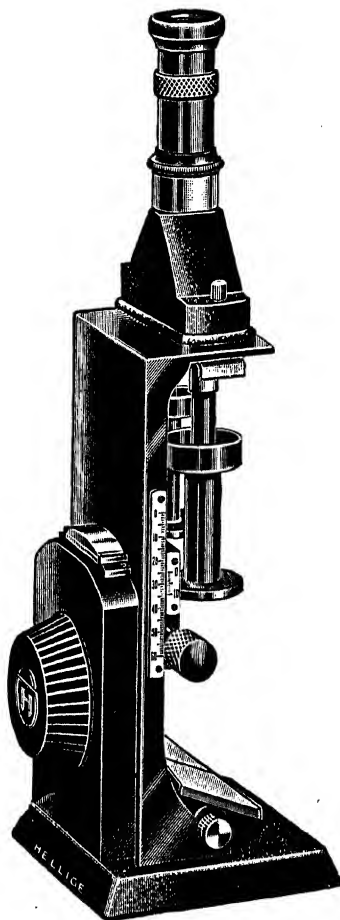


FIG. 37

Direct-Reading Duboseq Colorimeter.  
(Courtesy of Hellige, Inc.)

finely ground and in the best instruments are fused on or the plungers are of solid glass. In the less expensive instruments the bottoms are fastened with an adhesive.

The telescope, K, for observation of the colors is perpendicular to the

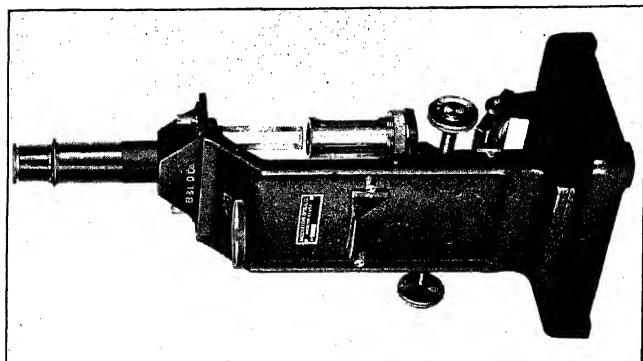


Fig. 39

Duboseq Colorimetric With Magnifier Scale Which Can Be Read from Observing Position. (Courtesy of Bausch & Lomb Optical Co.)

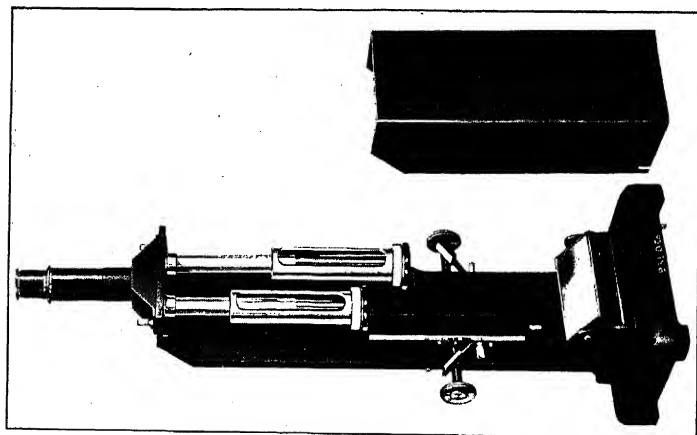


Fig. 38

Duboseq Colorimeter With Armored Cups, for Dilute Solutions. (Courtesy of Bausch & Lomb Optical Co.)

base so that the operator looks downward into the instrument. The light reflected upward through the solutions in A and B is so reflected by the prisms, I, I, in the box J that two fields appear side by side, one from A and one from B. The arrangement of the prisms is such that the images observed in the field of the telescope are those of the bottoms of the plungers, O, P, rather than of the entire depth of liquid in A and B. By suitable reduction of the aperture by screens, reflection from the sides of the tubes is cut off.

For use, place the instrument to face a source of light and adjust mirror G at the proper angle to reflect skylight upward through A and B. Fill both cups about half full of clear water and bring the plungers into contact with the water. On observing this through the eyepiece a circle with a rather indistinct line through the middle will be seen. There should be no detectable difference between the intensity of the two halves of the field. If there is a difference adjust the location of the instrument so that they are equally bright.

Place the cups in position and turn up until they just touch the bottom of the plungers. Set the scale so that the zero mark corresponds with this setting of the cups, or adjust the cup carriers so that the scales are at the zero marks when the cups just touch the bottoms of the plungers. Be sure that the cups are not interchanged after setting the zero point as the bottoms may not be of equal thickness.

Place in one cup a solution of standard and in the other the developed sample. Move the cup having the lighter color upward until the plunger just touches the surface of the liquid, or to a definite reading standardized for the method. Then move the other cup upward, observing its movement through the eyepiece, until the image of the base of the plunger in that liquid appears to be of the same intensity as that observed from the other field. The instrument is then balanced and the depths of liquid underneath the plungers have the same relation to each other as the total depths of liquid in A and B when the Campbell-Hurley instrument is balanced. The slits in which the holders of A and B move are calibrated so that the depths of liquid may be read directly and errors in reading depths of liquid in glass are eliminated.

As a further method of elimination of side light it is desirable to surround the cups with a suitable shield, furnished with many of the instruments, or to use cups with opaque sides. Modifications of this apparatus differing in details of construction are available from various manufacturers.

With this type of instrument, the nature of the color is not important,

provided it is apparently the same in sample and standard, as only the intensities are compared.<sup>13</sup> Many types of spectrophotometric measurements are inferior in analytical accuracy. By simple modification the same instrument is suitable for colorimetry, nephelometry and bichromatic pH work.<sup>14</sup>

**Modifications of Duboscq Colorimeter**—The optical system of the Duboscq colorimeter has been improved by a biprism to refract the rays from prisms over <sup>15</sup> the cups, and the scale of the Duboscq type of colorimeter has been so located as to be observed through a parallel eyepiece of the same focal length, beside that used for comparison of the standard and sample. Reading by a vernier, and a micrometer method of setting the zero point are also provided.<sup>16</sup> A similar system of reading has also been incorporated with a built-in scale and magnifier.

In a special type, Figure 40, for blood chemistry, the depth of the unknown is adjusted with a calibrated glass plunger <sup>18</sup> from which results are read directly by reference to a set of charts. In another, shown in Figure 41, the scale reads directly as the ratio between sample and standard. This avoids the necessity of setting the standard at a definite depth since the depth of either is immaterial provided the ratio between them is known.

For comparison with a bichromatic solution, as in pH work, it may be necessary to vary two colors. Suitable equipment has been

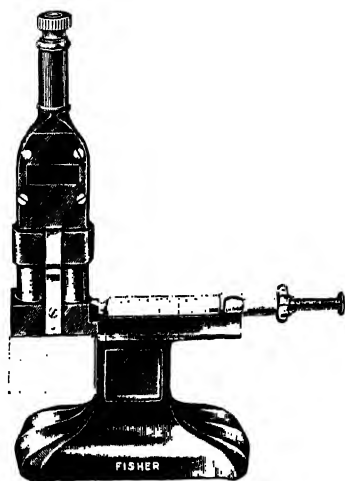


FIG. 40

The Vim-Sheftel Colorimeter.  
(Courtesy of Fisher Scientific Co.)

<sup>13</sup> E. Canals and E. Cabanes, *Bull. soc. chim. biol.* 14, 238-62 (1932).

<sup>14</sup> J. Grant and J. H. W. Booth, *J. Sci. Instruments* 10, 106-10 (1933).

<sup>15</sup> John W. Forrest, U. S. Patent 1,806,621 (1931).

<sup>16</sup> Robert E. Klett, U. S. Patent 1,535,070 (1925); *J. Biol. Chem.* 47, 19-25 (1921).

<sup>18</sup> Abraham G. Sheftel, U. S. Patent 1,916,589 (1933).

<sup>19</sup> G. R. Mines, *J. Physiol.* 40, 327 (1910).

<sup>20</sup> Louis J. Gillespie, *J. Bact.* 6, 399 (1921).

<sup>21</sup> Carl Zeiss, Inc., British Patent 280,552 (1928).

and is represented in Figure 42.<sup>22</sup> A and B represent the conventional fixed plungers. C and D represent containers for sample and standard. Both are fixed. E is a movable container for another form of standard. An important application is for pH determination with bichromatic indicators. The hydrogen-ion type of Duboseq colorimeter has two cells for the standard, of which one fits inside the other. The acid form of the indicator is used in either B or C and the alkaline form in the

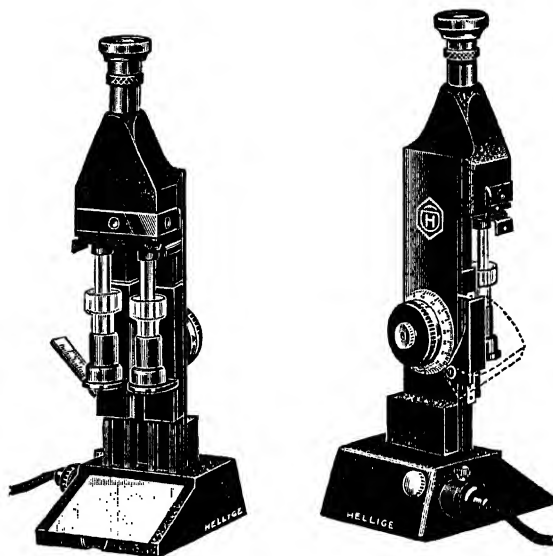


FIG. 41

Colorimeter Reading Directly as Ratio of Sample and Standard. (Courtesy of Hellige, Inc.)

other. D is filled with the sample solution. By variation of E the effective depth in C is also varied and the total depth in C and E is equal to the depth in D. Good results with phenol red were reported.<sup>23,24</sup> As another example a known amount of blood in urine in the outer cup, and urine without blood in the inner cup are matched against a sample of urine for estimation of the blood content. A similar principle has been utilized with one color of fixed depth and the other of variable depth.<sup>25</sup>

Another modified form<sup>26</sup> of the Duboseq instrument provides for a

<sup>22</sup> For more complete details see Figure 105, p. 702.

<sup>23</sup> H. Wu, *Proc. Soc. Exptl. Biol. Med.* 21, 111-4 (1923).

<sup>24</sup> M. Caille, *Bull. Soc. chim. biol.* 10, 590-601 (1928).

<sup>25</sup> E. Fuld and E. Schlesinger, German Patent 235,541 (1910).

<sup>26</sup> Jan Szczepanik, British Patent 22,995 (1907); German Patent 191,738 (1906).

nest of three cells, each independently movable and each containing a solution of a different color. A plunger is provided for the upper cell, the bottom of the upper and middle cells serving as plungers for those below. By this instrument any of three different colored liquids may be adjusted so as to match the sample in terms of three components of color.

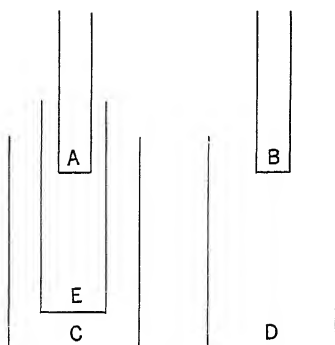


FIG. 42

Diagrammatic Arrangements of  
Duboseq Type of Hydrogen-  
Ion Colorimeter

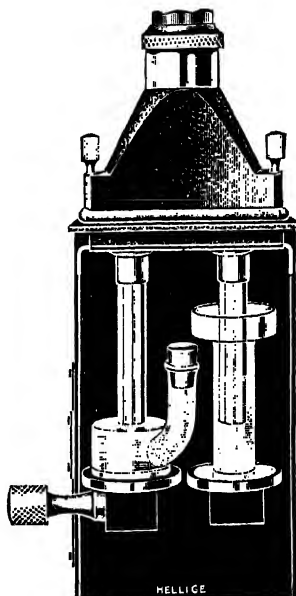


FIG. 43

Cell of Standard of Constant  
Depth for Duboseq Colori-  
meter. (Courtesy of  
Hellige, Inc.)

A special cell for use with the Duboseq type of colorimeter standardizes the depth of liquid standard, thus eliminating a possible error in adjustment. It is shown in Figure 43. For nephelometric use a fixed cell beside the usual movable cup is provided. Reflection is by mirrors instead of prisms.<sup>28,29</sup> Other improvements have been developed.<sup>30,31,32</sup>

<sup>28</sup> Joseph C. Bock and Stanley R. Benedict, U. S. Patent 1,456,964 (1923); *J. Biol. Chem.* 35, 227-30 (1918).

<sup>29</sup> Klett Mfg. Co., New York, N. Y.

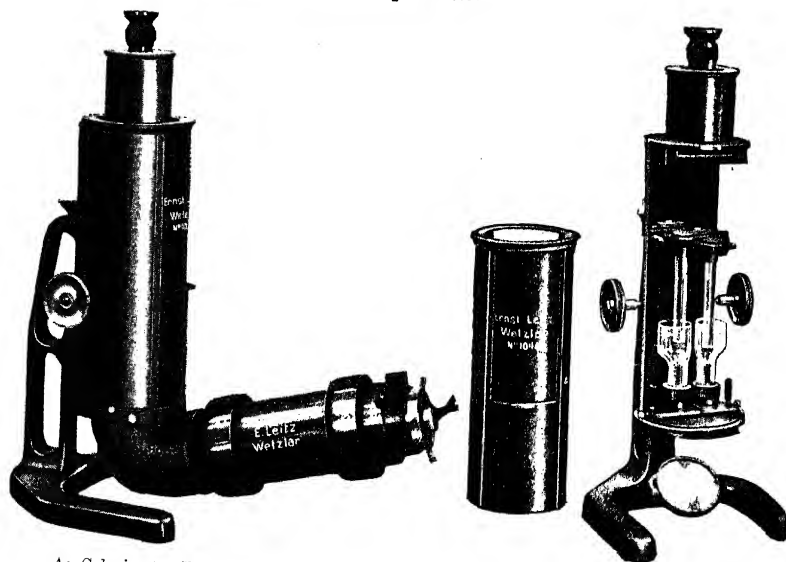
<sup>30</sup> W. L. Patterson, U. S. Patent 1,522,592 (1925).

<sup>31</sup> Hans Kleinmann, *Biochem. Z.* 179, 301-3 (1926).

<sup>32</sup> Gustave Fassin, U. S. Patent 1,939,547 (1933).



As Nephelometer



As Colorimeter With Artificial Illumination

As Duboseq Colorimeter

FIG. 44

Three Forms of Use of Universal Colorimeter. (Courtesy E. Leitz, Inc.)



The Duboseq colorimeter has been adapted to measurement of color of dyed fabric<sup>33</sup> and even to estimation of interfacial tension.<sup>34</sup> A recent type of instrument combines the Duboseq and Buerker methods with artificial lighting and the nephelometer. It is known as the Universal Colorimeter for Research.

**Microcolorimetry**—For microchemical work capillaries 0.2-0.5 mm. in diameter have been used. By use of such capillaries quantities determined in millionths of a mg. are: methylene blue 2.5, nitric acid by diphenylamine 10, colloidal gold 3, permanganic acid 15, manganese by the lead dioxide method 80, iron by thiocyanate 2.5, platinum with potassium iodide 5, acid or alkali with litmus 4.<sup>35,35a</sup>

The micro Duboseq colorimeter is a miniature of the usual instrument with cups of 1 cc. capacity. Accuracy to 1 per cent is attained, limitation being more in other details than in the apparatus.<sup>36</sup> A similar instrument with 2 cc. cups has been described.<sup>37</sup>

**Stammer Colorimeter**—The Stammer colorimeter is also a modified form of Duboseq, and is particularly used for determination of the color of sugar solutions and of oils. The colors of the fields are transmitted to the telescope, K, by prisms, I, I, as in the Duboseq type. The alteration is in the character of the fields. Instead of two movable containers and two fixed pistons as in the other instrument the containers are fixed and one movable piston, P, is provided for the variation of the column in B. A false piston, O, is provided so that the light through A will have to pass through a similar thickness of glass. The column in A remains permanent. Light is reflected upward by a mirror at G as in previous instruments.

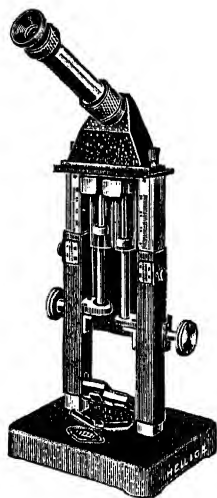


FIG. 45

Micro Duboseq Colorimeter.  
(Courtesy of Hellige,  
Inc.)

<sup>33</sup> H. S. Busby, *Textile World* 63, 1827-9 (1923).

<sup>34</sup> Frederick S. Hammett, *Science* 54, 172-3 (1921).

<sup>35</sup> F. Enrich and J. Donan, *Tech. Hochsch. Graz. Monatsh.* 28, 825-30 (1907).

<sup>35a</sup> Cf. A. A. Guntz, *Chemie & industrie*, Special No. 236-7 (April, 1934).

<sup>36</sup> H. Kleinmann, *Chem. Fabr.* 1928, 263-4, 278-9; *Biochem. Z.* 179, 276-86 (1926).

<sup>37</sup> A. Baudouin and H. Benard, *Compt. rend. soc. biol.* 83, 602-3 (1920).

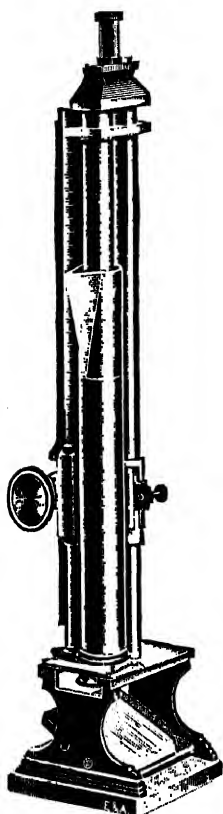


FIG. 46

Stammer Colorimeter. (Courtesy of  
Eimer & Amend)

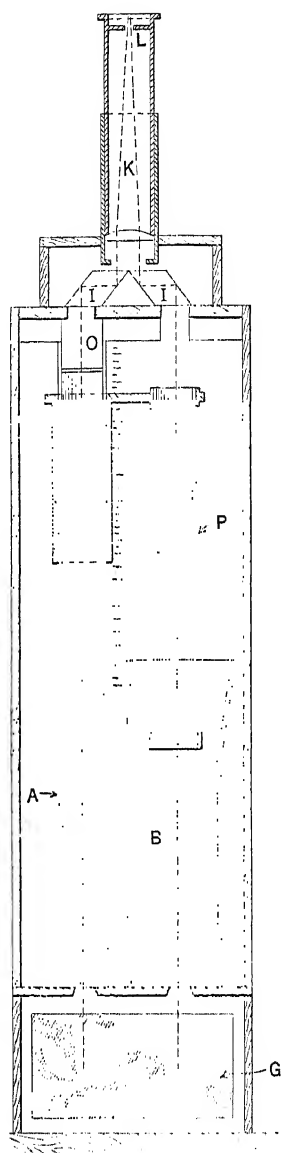


FIG. 47

Schematic Diagram of Stammer  
Colorimeter

Modifications<sup>38</sup> and artificial illumination<sup>39</sup> have been discussed. In a special type the tube can be filled, emptied and washed without removal from the instrument.<sup>40</sup> Results have been plotted in terms of readings of red and green filters.<sup>40a</sup>

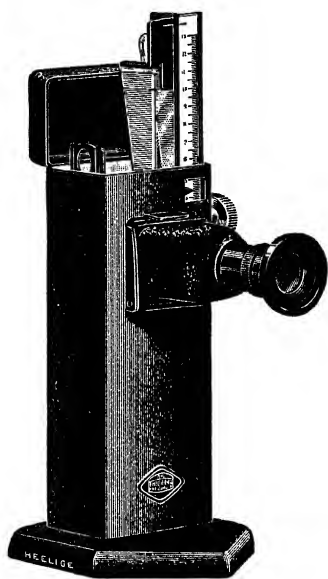


FIG. 48

Wedge-Type Colorimeter

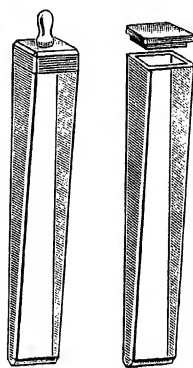


FIG. 49

Colorimeter Wedges



FIG. 50

Double Wedge for Standard in pH Determination.

(Courtesy of Hellige, Inc.)

**Wedge Type Colorimeters**—Another method of varying the depth of solution through which light is transmitted is to use a hollow wedge which can be moved vertically by a rack and pinion and give a direct scale reading.<sup>41-47</sup> In principle this might be compared with the unknown

<sup>38</sup> N. Roberts, *Bull. Hyg. Lab. P. H. and M. H. Serv.* 66, 3 (1910).

<sup>39</sup> V. Sazavsky, *Listy Cukrovarnické* 47, 137 (1928).

<sup>40</sup> W. Kopperl, *Deut. Zuckerind.* 56, 1037 (1931).

<sup>40a</sup> Brukner and Becker, *Deut. Zuckerind.* 59, 692-3 (1934).

<sup>41</sup> Charles H. White, U. S. Patent 840,538 (1907); *J. Am. Chem. Soc.* 34, 659-62 (1912).

<sup>43</sup> Anon., *Chem.-Ztg.* 36, 747 (1912); *Z. angew. Chem.* 25, 1563-4 (1912).

<sup>44</sup> G. D. Barnett and C. W. Barnett, *Proc. Soc. Exp. Biol. Med.* 18, 127 (1921).

<sup>45</sup> V. C. Myers, *J. Biol. Chem.* 54, 675 (1922).

<sup>46</sup> I. M. Kolthoff, *Rec. trav. chim.* 43, 144 (1924).

<sup>47</sup> J. McCrae, *Analyst* 51, 287 (1926).

in a cell of fixed thickness or in a similar wedge. Numerous models of this type of instrument have been devised but none seem to have become as popular as the Campbell-Hurley or Duboseq instruments.

The apparatus consists essentially of 2 wedge-shaped hollow glass prisms of equal dimensions, open at the large end for the introduction of the solutions to be tested. The wedges are held in vertical position side by side in a camera and may be raised or lowered by a rack and pinion actuated by thumbscrews. The wedges are screened from view on the side of the operator, except for a narrow horizontal slit across the middle of the camera through which the solutions are observed when a determination is being made. The carriers are graduated to correspond to the length of the wedges, the zero of the scale being opposite the indicator when the sharp edge of the wedge is opposite the narrow opening in the screen through which the color is observed. The screens are adjustable so that the opening may be altered to suit the operator. The ground glass shutter at the forward end of the camera, for diffusing the light, is hinged as a door to facilitate the transfer of the wedges to and from the camera. To carry out a determination by this method the wedges are filled with standard and sample solutions. The wedge containing the solution of unknown strength is set at the graduation representing the percentage, or a multiple of it, of the coloring matter in the standard. The wedge containing the standard is then adjusted so that the two fields seen through the camera appear identical. The percentage of coloring matter in the unknown is then indicated by the reading of the scale on the carrier containing the standard. If the depths of color compared at first are too dark or too light for accurate comparison, the results may be checked without changing the solutions by setting the wedge containing the unknown at a new point on the scale and again adjusting the other wedge until a balance is obtained. The maximum error of reading reported on a long series of experiments with this instrument was 0.6 per cent.

The instrument is claimed to cause less eye fatigue than the usual types because the eye is rendered sensitive to color by light rather than by darkness. The operator cannot see the scale while operating and therefore tends toward greater accuracy. Checks at different color intensities without changing either solution are an advantage.

The Autenrieth-Koenigsberger colorimeter compares a colored standard solution in a wedge shaped vessel with a sample in a rectangular container. The graduated scale is calibrated for the particular substance

to be determined by comparison with known solutions.<sup>48,49</sup> It has been applied to numerous determinations, both inorganic and biological.<sup>50,51</sup>

**Wedge Type Bi-Colorimeter**—The wedge type of colorimeter has been applied to two-color solutions.<sup>52-55</sup>

In ordinary colorimetry only one wedge of standard is necessary. For bicolorimetry such as estimation of pH, two wedges are used, or a double wedge becomes necessary, Figure 50. When the original solution is colored or turbid a third wedge containing the solution without the test color developed is used for compensation.<sup>56</sup> It is also suited to microcolorimetry in a modified form.<sup>57</sup> To compensate for the greater thickness of glass with the standards than with the sample additional glass plates are affixed to the cell containing the sample. In use the wedges may be calibrated to read directly in percentage, with solutions which follow Beer's law. Readings in pH have been obtained with an accuracy of 0.02 unit.

**Miscellaneous Wedge Types**—Difficulty with a wedge type of instrument with higher alcohols has been reported as due to different refraction in two different shaped observation vessels. This was overcome by immersing both in 60 per cent sulfuric acid.<sup>58</sup> A wedged shaped tube is provided in a design for blood work.<sup>59</sup> The sample required is very small. A special type in which unknowns in test tubes are read against a calibrated wedge of standard has been described with particular reference to the fibrin-carmin method for pepsin.<sup>60</sup>

Another wedge type compares the sample with wedges of green, red, blue and neutral grey,<sup>61</sup> thus matching any hue or degree of saturation. Duplication of any color in terms of colored wedges is provided for in

---

<sup>48</sup> W. Autenrieth and Albert Funk, *Z. anal. Chem.* 52, 137-67 (1913).

<sup>49</sup> Hans Magnus, *Deut. med. Wochschr.* 55, 1803 (1929).

<sup>50</sup> W. Autenrieth and J. Koenigsberger, *Munch. med. Wochschr.* 57, 998-1001 (1910).

<sup>51</sup> H. Schnegg and W. Wollmer, *Z. ges. Brauw.* 38, 33-5, 41-3 (1915).

<sup>52</sup> V. C. Myers, *J. Biol. Chem.* 54, 675 (1922); *Proc. Soc. Exptl. Biol. Med.* 19, 78 (1921-2).

<sup>54</sup> G. D. and C. W. Barnett, *Proc. Soc. Exptl. Biol. Med.* 18, 127 (1920-21).

<sup>55</sup> E. Leitz, Inc., New York, N. Y.

<sup>56</sup> V. C. Myers, *J. Biol. Chem.* 55, 569 (1923).

<sup>57</sup> V. C. Myers, *J. Lab. Clin. Med.* 7, 237-9 (1922).

<sup>58</sup> E. Mullershossly, *Mitt. Lebensm. Hyg.* 9, 85-6 (1918).

<sup>59</sup> Robert E. Klett, U. S. Patent 1,375,708 (1921).

<sup>60</sup> P. v. Grutzner, *Arch. ges. Physiol.* 144, 545-54 (1912).

<sup>61</sup> Lloyd A. Jones, U. S. Patent 1,496,374 (1924).

another patent.<sup>62</sup> The intensity of grey or color with wedges is measured in a simple reflection instrument.<sup>63</sup>

**Special Prism Type**—A prism type is stated to be the most accurate yet devised.<sup>64</sup> It is illustrated in Figure 51. The white light from a frosted lamp, L, illuminates two diaphragms B, B', with equal intensity.

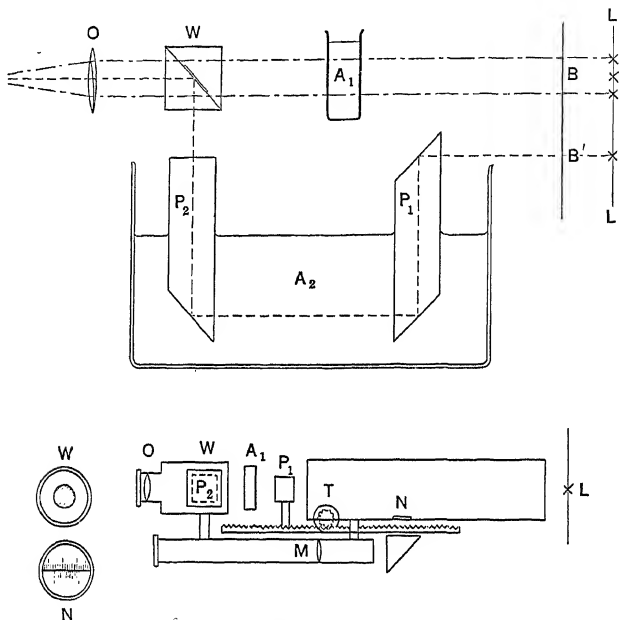


FIG. 51

Special Prism Type of Colorimeter

From one of these, the light passes through a small glass receptacle, A', usually containing the standard solution, and from thence, through the periphery of a photometric cube, W, into the ocular of the instrument, O. The light from the lower diaphragm, B', strikes against a prism, P<sub>1</sub>, passes through another liquid, A<sub>2</sub>, usually the solution to be analyzed, and thence to another prism, P<sub>2</sub>, from which it strikes a mirror in the middle of the photometer cube, W, and is reflected into the ocular of the instrument. The prisms, P, P', are fastened to a sliding scale so that the distance between them can be regulated at will. By moving one prism back and

<sup>62</sup> Victor Herbert Gregory, British Patent 15,678 (1912).

<sup>63</sup> Edward Sanger-Shepherd, British Patent 23,429 (1911).

<sup>64</sup> A. L. Bernouilli, *Helv. Chim. Acta* 9, 827-40 (1926).

forth it is easy to obtain an adjustment such that the two shades of color resulting from the absorption of light by the two liquids match. No special vessel is required to contain the solution to be analyzed. A beaker or evaporating dish is suitable. By placing the same liquid in both vessels, the exact position on the scale, which corresponds to the same concentration of solutions, for the given color, is determined. This position is independent of the concentration but varies somewhat with different colors of solution. From the position when the sample solution is used, it is easy to estimate the concentration.

**Photoelectric Colorimeters**—Colorimeters have been constructed in which the transmitted light is measured by its effect on a photoelectric cell.<sup>65-86d</sup> The principle is either to compare standard and sample utilizing

<sup>65</sup> Wilhelm Berg, German Patent 296,050 (1911).

<sup>66</sup> S. P. Reimann, *Proc. Soc. Exp. Biol. Med.* 23, 520-3 (1926).

<sup>67</sup> L. B. Desbleck, *Electrician* 99, 8 (1927).

<sup>68</sup> C. H. Sharp and C. Kinsley, *Elec. World* 87, 823 (1926).

<sup>69</sup> E. Cuboni, *Bull. inst. sieroterap. milanese* 1927, No. 6.

<sup>70</sup> Karl S. Felix and Hermann Wanderscheck, German Patent 529,309 (1927).

<sup>71</sup> Charles N. Race, U. S. Patent 1,739,373 (1929).

<sup>72</sup> H. Reeve Angel & Co., *Silk J.* 6, 43-4 (1930).

<sup>73</sup> K. Sandera, *First Communications New Intern. Assoc. Testing Materials* (Zurich) 1930, Group D, 225-7.

<sup>74</sup> Frank Twyman and John Perry, U. S. Patent 1,775,148 (1930).

<sup>75</sup> A. Dolínek, *Listy Cukrovar.* 49, 203-5 (1930); *Z. Zuckerind. Czechoslovak Rep.* 55, 376-8 (1931).

<sup>76</sup> Samuel Wein, U. S. Patent 1,779,574 (1931).

<sup>77</sup> Walter M. Scott, *Textile World* 79, 886-9 (1931).

<sup>78</sup> O. Spengler and E. Landt, *Z. Ver. deut. Zuckerind.* 81, 13-24 (1921).

<sup>79</sup> C. Digand, *Ann. chim. anal. chim. appl.* 13, 1-5, 33-54, 65-72 (1931).

<sup>80</sup> Arthur C. Hardy and Frederick W. Cunningham, U. S. Patents 1,806,197, 1,806,198, 1,806,199 (1931).

<sup>81</sup> Ralph H. Müller and Herman M. Partridge, *Ind. Eng. Chem., Anal. Ed.* 3, 167-71 (1931).

<sup>82</sup> J. Razeq and P. J. Mulder, British Patent 349,565 (1929).

<sup>83</sup> F. C. Whalen and Radiovisor Parent, Ltd., British Patent 350,703 (1930).

<sup>84</sup> Bruno Lange, *Chem. Fabrik* 5, 457-9 (1932); *Physik. Z.* 31, 964-9 (1930); *Naturwissenschaften* 19, 103-7, 128-32 (1931); German Patent 546,676 (1930); *Schrecksaal* 65, 255-6, 275-7, 293-5 (1932).

H. Müller, *Mikrochemie* 10, 285-96 (1931).

J. V. Wilcox, *Ind. Eng. Chem., Anal. Ed.* 6, 167-9 (1934).

<sup>86a</sup> R. Fonteyne and P. de Smet, *Mikrochemie* 13, 289-304 (1933).

<sup>86b</sup> Leonard T. Troland, U. S. Patent 1,971,737 (1934).

<sup>86c</sup> Martin W. Baden, U. S. Patent 1,938,004 (1934).

<sup>86d</sup> Ch. Zinzadze, *Ind. Eng. Chem., Anal. Ed.* 7, 280-1 (1935).

photoelectric cells to determine the balance, or to measure the transmittance of a standard depth of sample solution and compare with a calibration curve. The future applications of this type of equipment are limited only by the desire for simplicity, as contrasted with the greater accuracy obtainable. Published descriptions relate to malt, worts and beer,<sup>87,88</sup> to hemoglobin<sup>89,90,91</sup> and to petroleum,<sup>92</sup> as typical materials.

By proper choice of filter, absorption-cell thickness and concentration of the solution, Beer's law can be satisfied and measurement greatly simplified.<sup>93</sup> While the vacuum photoelectric cell-amplifier method gives the highest accuracy, barrier-layer cells are precise and simpler.<sup>93a</sup> The methods are applicable to macro or micro work. Copper in brass is estimated by this method with the same degree of accuracy as by titration. Accuracy to 1 per cent is obtained for chromium in steel.<sup>93b</sup> Poor definition of color will limit the accuracy with many solutions.<sup>93c</sup> Satisfactory accuracy is attained in comparison of solutions of nearly equal concentration.<sup>94</sup> The equipment is not necessarily expensive for reasonably accurate results.<sup>94a</sup> The cuprous oxide photoelectric cell has also been applied to nephelometry<sup>95</sup> and another form to turbidimetry.<sup>96</sup>

**Yoe Photoelectric Colorimeter**—Since the trends indicate the future commercial use of such a colorimeter as the Yoe photoelectric colorimeter, this unit is described in detail.<sup>96a</sup> The unit is compact, portable and commercially available at a moderate price. The complete instrument is shown in Figure 52, and the circuit in Figure 53.

*Photocell Circuit.* Only one photoelectric cell is used. It is of the

<sup>87</sup> Karl S. Felix, Helmrich and Wanderscheck, *Wochschr. Brau.* 45, 312-6 (1928).

<sup>88</sup> F. Stockhausen and F. Windisch, *Ibid* 45, 231 (1928).

<sup>89</sup> F. Wildermuth, *Arch. ges. Physiol.* 183, 91-108 (1920).

<sup>90</sup> G. A. Millikan, *J. Physiol.* 79, 152-7 (1933).

<sup>91</sup> T. Kofman, *Compt. rend. soc. biol.* 112, 481-2 (1933).

<sup>92</sup> B. W. Story and V. A. Kalichevsky, *Ind. Eng. Chem., Anal. Ed.* 5, 214-17 (1933)

<sup>93</sup> Ralph H. Müller, *Mikrochemie* 11, 353-68 (1932).

<sup>93a</sup> Ralph H. Müller, *Ind. Eng. Chem., Anal. Ed.* 7, 223-6 (1935).

<sup>93b</sup> A. L. Davindov and V. F. Stefanovski, *Zavodskaya Lab.* 3, 640-5 (1934).

<sup>93c</sup> A. H. W. Aten, N. Galema and C. A. Goethals, *Chem. Weekblad* 31, 258-64 (1934).

<sup>94</sup> E. Canals and A. Hortalá, *Bull. soc. chim. biol.* 14, 1273-8 (1932).

<sup>94a</sup> Arthur Weil, *Science* 79, 593 (1934).

<sup>95</sup> T. Kofman, *Compt. rend. soc. biol.* 112, 478-80 (1933).

<sup>96</sup> G. Gollnow, *Gas u. Wasserfach* 75, 848-9 (1932).

<sup>96a</sup> John H. Yoe and Thomas B. Crumpler, *Ind. Eng. Chem., Anal. Ed.* 7, 281-4 (1935).



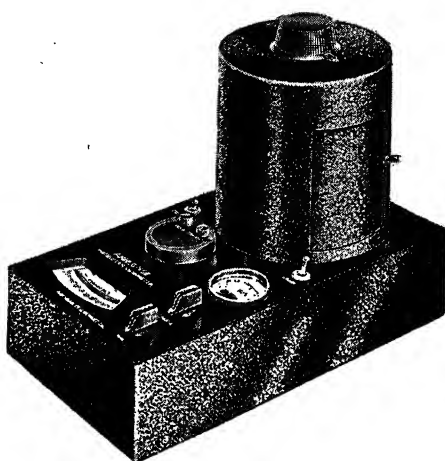


FIG. 52

Yoe Photoelectric Colorimeter  
(Courtesy of American Instrument Co.)

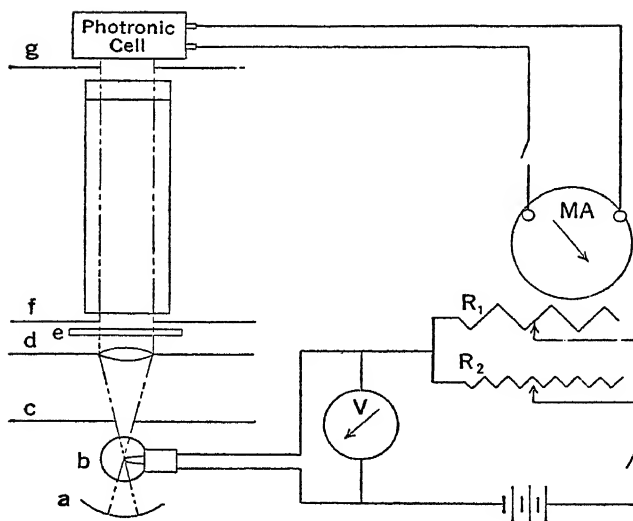


FIG. 53

Diagram of Yoe Photoelectric Colorimeter

photovoltaic type which acts as a source of current without the aid of an external e.m.f. The fundamental characteristic of this type of cell is the current which is almost exactly proportional to the light intensity for low resistances in the external circuit, rather than the e.m.f. as is usual in the ordinary sources of electrical energy. The photronic cell<sup>96b</sup> is connected in series with a microammeter, MA, having a resistance of 50 ohms and reading up to 50 microamperes. By the use of a special scale this reads to 0.1 microampere.

*Light Circuit.* The source of energy is a 6 volt, 17 plate lead storage battery. The lamp is a 6 to 8 volt single filament bulb such as is used in automobile headlights. This lamp, *b*, is connected through resistances,

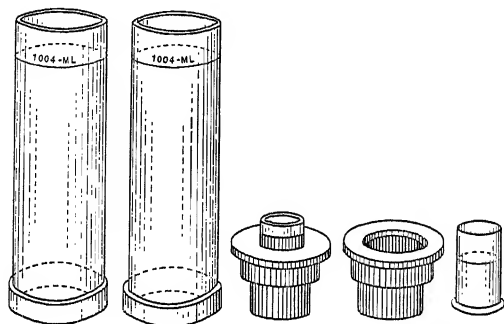


FIG. 54

Tube and Adapters for Yoe Photoelectric Colorimeter

$R_1$ ,  $R_2$ , in parallel. One is for coarse adjustment, the other for fine adjustment. A small voltmeter,  $V$ , across the lamp reads up to 8 volts and permits approximate adjustment of the resistances.

*Optical System.* The adjustable spherical metal reflector, *a*, below the lamp has the filament at its center of curvature. The light rays are therefore reflected back upon themselves to approximately double the intensity of the beam traveling upward through the diaphragm, *C*, which limits the beam. The lens, *d*, has a diameter of 2.5 cm. and is placed at its focal length, 5 cm., so as to render the beams striking it nearly parallel. The lamp is adjustable to permit centering with respect to the lens. The beam is further limited by another diaphragm, *f*, before it enters the tube of liquid. The unabsorbed light is again limited by another diaphragm, *g*, before striking the photocell. These precautions minimize stray reflec-

<sup>96b</sup> Model 594, Weston Electrical Instrument Corporation, Newark, N. J.

tions from the sides of the tubes, lens-effect of drops at the tops of the tubes, reflections from fingerprints on the outside of the tubes and the lens-effect of the meniscus. The latter is largely eliminated by having the beam small in comparison with the diameter of the meniscus.

*Tubes.* The tubes, Figure 54, are of clear glass, precision-bore tubing. The bottoms are optically plane to prevent distortion of the beam and fused on. The design calls for a 100 cc. content to the 100 mm. mark. A brass ring with a slot in it is cemented to the bottom of each tube. This coincides with pins in the housing so that the tubes are always in the same position. Two tubes are mounted on a turntable which rotates them into position in the path of the beam. Two fixed points mark the correct position of the ratchet-actuated turntable. A slide provides for insertion of color filters over the lens, *e.* In the absence of a filter an optical flat protects the lens from dust. The sliding door on the side of the housing closes tightly to keep out stray light and dust. Adapters can be mounted on the turntable from the shoulders that align the tops of the large tubes. These adapters hold 5 cc. tubes for micro work.

*Method of Use.* Before use, fill the clean tubes to the mark with distilled water and place in position. Close the sliding panel of the instrument and turn on the light switch. Adjust the coarse resistance to about 3 volts. Allow 2-3 minutes for filament and rheostats to warm up and assume constant resistance. Close the photocell switch and adjust the light intensity to a reading of 50 on the microammeter with tube I in position. Quickly turn tube II into position, and the reading should adjust itself again to 50. Turn tube I back to insure accuracy. Repeat until 3 comparisons are obtained. First readings may be discarded. If the tubes do not balance, carefully clean the bottoms.

For use, fill tube I with the blank solution and adjust the microammeter to 50. This blank contains all color-producing substances except that from the test substance. Correction is thus made automatically. A sensitive voltmeter is not required as the voltage need not be measured.

This equipment has a defect in the "fatigue" effect of the photonic cell. When first exposed to light it reads high, but shortly drops to a constant value. When the difference between sample or standard and blank is small, this effect is negligible. With highly colored solutions, allow a few minutes for adjustment before reading the blank. This may permit a voltage change across the lamp. For high accuracy, therefore, have standard and sample of similar concentration.

Results are then obtained by the equation 
$$-\frac{\log 50/R_1}{\log 50/R_2}$$
 and  $R_2$  are the ammeter readings for  $c_1$  and  $c_2$ .

Color filters may be used to eliminate effects of colored reagents or non-reacting substances. For microtubes use a reading of 25 for the blank instead of 50 to avoid rapid discharge of the battery. Results may be plotted in terms of ammeter readings against concentrations. Results may be read directly and preclude error due to the fatigue effect of the cell. If Beer's law holds, decrease in ammeter readings may also be plotted against concentration. If the blank shows no absorption or can be corrected by a filter, use distilled water in tube I for convenience. Substantially higher accuracy was obtained in estimating cupric-ammonium ion than by visual comparison, an accuracy estimated at 5-10 times as good. In consideration of the use of such instrument for any particular estimation, the probable errors in the method other than those in comparing colors must be considered.

**Special Types and Uses**—Types for special conditions have been devised such as for strongly acid solutions,<sup>97</sup> elevated temperatures,<sup>98</sup> double color work,<sup>99</sup> reading stained histological sections,<sup>99a</sup> absolute colorimetry without use of a standard,<sup>100,101,101a,101b</sup> determination in the presence of a colored medium,<sup>102</sup> high precision,<sup>102a</sup> luminescence,<sup>102b</sup> oxidation-reduction studies,<sup>102c</sup> and for corrosive gases.<sup>103</sup>

Among types for special purposes are those for dextrose,<sup>104</sup> turpentine,<sup>105</sup> milk<sup>106</sup> and its acidity,<sup>107</sup> bilirubin,<sup>108</sup> hemoglobin<sup>109</sup> micro methods,<sup>110</sup> pH and turbidity of sewage or other colored or turbid

<sup>97</sup> Th. von Fellenberg, *Mitt. Lebensmittelfinters. u. Hyg.* 1, 351-2 (1911).

<sup>98</sup> A. Scholtz, *Chem.-Ztg.* 38, 497-8 (1914).

<sup>99</sup> E. Kaufmann, *Biochem. Z.* 197, 141-2 (1928).

<sup>99a</sup> Edward G. Kelley, *J. Biol. Chem.* 110, 141-4 (1935).

<sup>100</sup> Raymond Defay, *Bull. soc. chim. biol.* 8, 733-45 (1926).

<sup>101</sup> A. Thiel and W. Thiel, *Chem. Fabrik* 1932, 409-11.

3. Guelke, *J. Soc. Dyers Colorists* 50, 77-80 (1934).

I. Riffart, *Z. Untersuch. Leberns m.* 68, 566-7 (1934).

<sup>102</sup> W. Lampe, *Deut. med. Wochschr.* 51, 181-2 (1925).

<sup>102a</sup> John J. Manley, *Proc. Phys. Soc. (London)* 47, 69-73 (1935).

<sup>102b</sup> Rudolf Seifert, *Apoth. Ztg.* 50, 590-3 (1935).

<sup>102c</sup> Harold P. Lundgren, *Science* 80, 209 (1934).

<sup>103</sup> E. C. White and R. C. Tolman, *Proc. Am. Phys. Soc., Phys. Rev.* 22, 207 (1923).

<sup>104</sup> Philip Atlee Sheaff, *J. Am. Med. Assoc.* 65, 1181-2 (1915).

<sup>105</sup> C. F. Sammet, *J. Ind. Eng. Chem.* 8, 519-21 (1916).

<sup>106</sup> Alexander Bernstein, *Chem.-Ztg.* 61, 727 (1908).

<sup>107</sup> Christian Hackmann, German Patent 503,245 (1927).

<sup>108</sup> E. Meulengracht, *Deut. Arch. klin. Med.* 1921, 38-46.

<sup>109</sup> Arthur T. Brice, Jr., U. S. Patent 1,859,278 (1932).

<sup>110</sup> Ferdinand Lebermann, *Munch. med. Wochschr.* 72, 982-5 (1926).

liquids,<sup>111</sup> automatic mixture of solution and indicator for pH control,<sup>112</sup> measuring the color of the skin, sugar work,<sup>113</sup> formol titra-

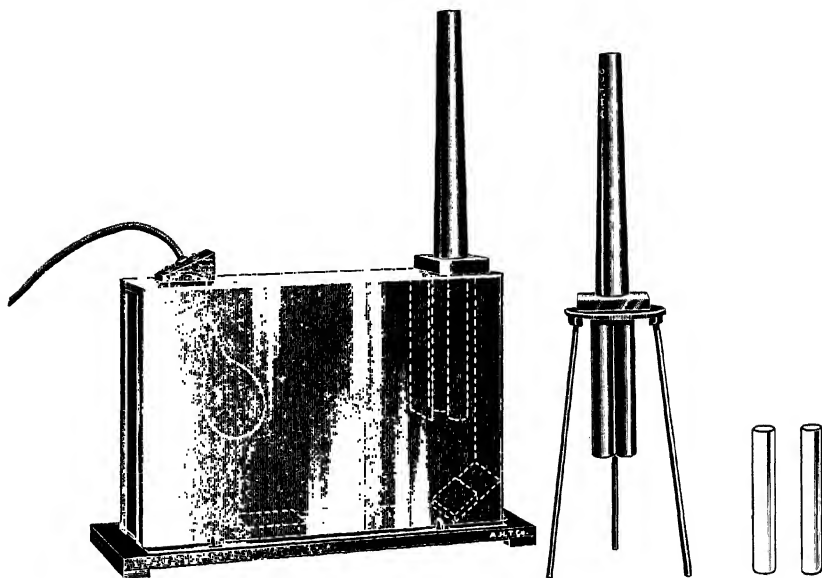


FIG. 55

Two Types of Wesson Colorimeter Used in Examination of Vegetable Oils. (Courtesy of A. H. Thomas Co.)

tion,<sup>115</sup> colorimetric titration,<sup>116,117</sup> tan liquors,<sup>118</sup> chlorine,<sup>119,119a</sup> manganese by periodate,<sup>120</sup> steel analysis,<sup>121</sup> and for oils in terms of red, yellow and neutral tints.<sup>122</sup>

<sup>111</sup> B. G. Peters, *J. Helminthology* 7, 201 (1929); *Centr. ges. Hyg.* 22, 443; *U. S. Pub. Health Eng. Abstracts* 11, S, 46 (1931).

<sup>112</sup> W. Kordatzki, *Chem. Fabrik* 1931, 485-6.

<sup>113</sup> A. E. Bawtree, *Intern. Sugar J.* 22, 556-7 (1920); *J. Oil Colour Chemists Assoc.* 4, 165-88 (1921).

<sup>115</sup> Ludwig Adler, *Z. ges. Brau.* 38, 241-3, 251-2 (1915).

<sup>116</sup> Edwin B. Powers, *Ecology* 9, 364 (1928).

<sup>117</sup> K. Mayer, *Biochem. Z.* 231, 314-6 (1931).

<sup>118</sup> C. C. Smoot, *J. Am. Leather Chem. Assoc.* 19, 571-4 (1924).

<sup>119</sup> W. Paterson, British Patent 314,155 (1928).

<sup>119a</sup> Gerald D. Peet, U. S. Patent 1,976,672 (1934).

<sup>120</sup> G. Frederick Smith and V. R. Sullivan, *J. Chem. Education* 9, 1461-71 (1932).

<sup>121</sup> Will C. Neahr, U. S. Patent 1,728,358 (1929).

<sup>122</sup> David Wesson, *Cotton Oil Press* 4, 64-7 (1920).

Calibration of pipets delivering 0.0005 to 1.0 cc. has been proposed by filling them with blood of known hemoglobin content. The volume delivered would then be estimated from the hemoglobin content of the blood delivered.<sup>123</sup>

Equilibria in desmotropisomeric diacetosuccinic acid esters<sup>124</sup> have been studied in the colorimeter. There are a pocket type<sup>125</sup> and an immersion type.<sup>126</sup> Among simple types<sup>127,128</sup> is the comparison of two tubes immersed in a beaker of water in a dark box with suitable openings.<sup>129</sup> The application of various types to oils and paints<sup>130</sup> has been reviewed, as have also new types.<sup>131,132,133,133a</sup> For oxides of nitrogen in air, evacuated tubes with capillaries are opened by breaking the capillary at a file mark. When filled with the gas to be examined, the opening is sealed with wax. To transfer the sample another file mark is made and the capillary with wax broken off. Transfer of the gas is carried out in the usual way under mercury.<sup>134</sup>

An automatic colorimeter is designed for control of chlorination by the benzidine-potassium iodide method.<sup>135</sup> Another instrument compares solutions in square graduated containers.<sup>136</sup> For measurement of either color or turbidity, the comparative effect of observation of a target through variable depths of a sample solution has been provided with a direct reading scale.<sup>137</sup> In special cases two solutions may be compared with a standard of unknown concentration, the standard being kept at a

<sup>123</sup> Franklin C. Bing, *J. Lab. Clin. Med.* 18, 531-4 (1933).

<sup>124</sup> H. P. Kaufmann, *Ber.* 55, 202-48 (1922).

<sup>125</sup> Theodore Kuttner, *J. Am. Med. Assoc.* 65, 245 (1915).

<sup>126</sup> K. Burkner, *Z. angew. Chem.* 36, 427-9 (1923).

<sup>127</sup> F. Meinek and Margarete Horn, *Kl. Mitt. Ver. Wasserversorg. Abwasser-beseitig.* 2, 130-5 (1926); *Chem. Zentr.* 1927, II, 1745.

<sup>128</sup> E. Moreau, *Ann. fals.* 10, 235-7 (1917); *J. Chem. Soc.* 112, II, 418 (1918).

<sup>129</sup> O. Mannebach, *Chem.-Ztg.* 46, 20 (1922).

<sup>130</sup> F. W. Shulenberger, *Paint, Oil and Chem. Rev.* 73, No. 18, 8-9; No. 19, 8-9 (1922).

<sup>131</sup> Paul Verbeek, *Z. angew. Chem.* 27, Aufsatz. 203-8 (1914).

<sup>132</sup> Hugo Freund, *Chem.-Ztg.* 50, 1941 (1926).

<sup>133</sup> Karl Wiesler, *Chem.-Ztg.* 51, 750 (1927).

<sup>133a</sup> Virgil A. Schoenberg, U. S. Patent 1,938,544 (1934).

<sup>134</sup> V. C. Allison, W. L. Parker and G. W. Jones, *Bur. Mines, Tech. Paper* 249 (1921).

<sup>135</sup> G. Baganz, *Chem. Gabr.* 1928, 358.

<sup>136</sup> C. Berger, *Rev. gen. chim.* 14, 141-6 (1911).

<sup>137</sup> William G. Exton, U. S. Patent 1,635,470 (1927); U. S. Patent 1,717,702 (1929); German Patent 523,302 (1927); British Patent 292,337 (1927); British Patent 323,556 (1929).

constant depth throughout the determination.<sup>138</sup> This has been applied to determination of dissolved carbon dioxide. The advantage over comparison of unknown and standard is doubtful.

Numerous modifications of apparatus have been described for hydrogen-ion work, in practically all cases using some form of liquid standard but using varying types of equipment.<sup>139-148</sup>

A new instrument for pH is based on the same principle as the polarimeter.<sup>149</sup> Monochromatic nitrophenol indicators are used. The reduction in intensity of a beam of light passed through the solution is dependent on the intensity of color, which depends on the pH value. The character of the color does not change with the intensity. An accuracy to 0.05 unit of pH is claimed, even in turbid or colored solutions. Another instrument making comparison with light filters is apparently based on the same principle.<sup>150</sup>

Even temperature-control apparatus is available, as well as many other types.<sup>151-160</sup>

---

<sup>138</sup> S. P. D. McLean and R. B. Denison, *S. African J. Sci.* 23, 253-7 (1926).

<sup>139</sup> B. M. Duggar and C. W. Dodge, *Ann. Missouri Botan. Gardens* 6, 61-70, 179-81 (1919).

<sup>140</sup> Ernest Van Alstine, *Soil Sci.* 10, 467-79 (1920).

<sup>141</sup> I. M. Kolthoff, *Z. Nahr. Genussm.* 41, 112-22 (1921).

<sup>142</sup> Ed. Moreau, *Bull. sci. pharmacol.* 31, 335-41 (1924).

<sup>143</sup> W. H. Wright, *J. Lab. Clin. Med.* 13, 182-4 (1927).

<sup>144</sup> Rene A. Legendre, German Patent 487,897 (1928).

<sup>145</sup> Felix Pellin, French Patent 670,534 (1928).

<sup>146</sup> Hans Kroepelin, *Biochem. Z.* 198, 225-32 (1928).

<sup>147</sup> D. McCandlish and G. Hagues, *J. Inst. Brewing* 35, 66-8 (1929).

<sup>148</sup> A. Thiel, *Sitzber. Ges. Beförder. ges. Naturw. Marburg* 65, 159-67 (1930).

<sup>149</sup> H. C. Prinsen-Geerligs, *Arch. Suikerind.* 38, 993-4 (1930); *Facts About Sugar* 26, 38 (1932).

<sup>150</sup> L. Hoek and H. T. Müller, *Z. wiss. Phot.* 29, 262-6 (1930).

<sup>151</sup> R. Legendre, *Compt. rend. soc. biol.* 113, 1031-4; *Compt. rend.* 196, 1875-7 (1933).

<sup>152</sup> B. A. Revzyuk, Russian Patents 29,622, 29,623 (1932).

<sup>153</sup> J. Ripert, British Patent 346,170 (1930).

<sup>154</sup> A. C. Hardy, British Patent 341,200 (1928).

<sup>155</sup> L. I. Demkina, Russian Patents 20,361, 26,094 (1932).

<sup>156</sup> Wm. L. Patterson, U. S. Patent 1,799,639 (1931).

<sup>157</sup> J. W. Perry, *Farben-Chem.* 3, 125-9 (1932).

<sup>158</sup> R. Czerny, *Wasser* 6, 155-67 (1932).

<sup>159</sup> Hans Schneider, *Boll. chim. farm.* 72, 519-30 (1933).

<sup>160</sup> Everett H. Bickley, U. S. Patent 1,914,322 (1933).

## CHAPTER VI

### ARTIFICIAL LIQUID STANDARDS

IN MANY cases the color produced by a reaction fades in a short time in the light. In such case either a standard must be prepared at the same time as the sample, and the comparison made quickly, or permanent artificial standards must be prepared. Artificial standards are also often used to duplicate standard colors of unknown composition. Such standards are prepared from solutions of inorganic salts, combining colors if necessary. A simple example is the use of potassium bichromate for determination of sugar by the picric acid method.

The idea is very old, the color of London water having been read in terms of artificial standards in 1881<sup>1</sup> and a platinum color standard proposed in 1892.<sup>2</sup> The latter is still included in the Methods of the American Public Health Association.

The most complete series of artificial standards are those of H. V. Army, originally worked out for caramel and tincture of cudbar but later adapted to general use.<sup>3-6</sup> Three separate series were developed.

Half normal acidified solutions of the nitrates or chlorides of cobalt, iron and copper may be combined in such a way as to form any color desired except the deep blues and reds. The accuracy of this selection of the acid iron standard has been confirmed.<sup>10a</sup> Solutions of ferric chloride 0.02–0.5 *M* containing 0.05–5.0 *N* hydrochloric acid are stable and suitable for such use, as shown by transmission curves.

It has been found that some of the missing colors can be obtained by using ammoniacal solutions with dichromate in place of iron. The remaining colors are obtained by combinations of potassium dichromate and

---

<sup>1</sup> William Crookes, William Odling and C. Maymott Tidy, *Chem. News* 43, 174 (1881).

<sup>2</sup> Allen Hazen, *Amer. Chem. J.* 14, 300-10 (1892).

<sup>3</sup> H. V. Army, *Amer. Druggist* 59, 35 (1912); *Proc. 8th Int. Cong. App. Chem.* 26, 319 (1912); *J. Am. Pharm. Assoc.* 2, 76-80 (1913); *Deutsch-Amerikanische Apotheker Zeitung* 33, 165 (1913).

<sup>4</sup> H. V. Army and E. G. Pickhardt, *Druggists Circ.* 57, 131 (1914).

<sup>5</sup> H. V. Army and C. H. Ring, *J. Franklin Inst.* 180, 199-213 (1915).

<sup>6</sup> H. V. Army and Abraham Taub, *J. Am. Pharm. Assoc.* 12, 839-49 (1923); *J. Franklin Inst.* 196, 858 (1923).

<sup>10a</sup> M. G. Mellon and C. T. Kasline, *Ind. Eng. Chem., Anal. Ed.* 7, 187-9 (1935).



potassium permanganate. A mixture of the acid series, cobalt three parts to iron nine parts, diluted with water, corresponds to the color given by Nessler's reagent in reaction with ammonia. Varied dilutions of this may be used for a series of standards, making each standard to correspond to the colors obtained by the use of a known amount of ammonia treated with the necessary reagents. In a similar manner permanent standards may be prepared for any determination, keeping in mind the fact that every standard must be checked against a known amount of the test substance to make sure of its accuracy.

**Cobalt-Iron-Copper Series**—In this series all solutions are half normal and contain 1 per cent of hydrochloric acid. The composition of the solutions follows:

*Cobalt.* 59.59 grams of cobalt chloride,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , per liter in 1 per cent hydrochloric acid.

*Iron.* 45.05 grams of ferric chloride,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , per liter in 1 per cent hydrochloric acid.

*Copper.* 62.43 grams of copper sulfate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , per liter in 1 per cent hydrochloric acid.

These solutions are stable for at least two years.

**Cobalt-Chromate-Copper Series**—For this 0.02 *N* solutions in 2.8 per cent ammonium hydroxide are used.

*Roseo-Cobalt Chloride.* Dissolve 12 grams of cobalt chloride,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , in about 100 cc. of water. Filter if necessary and add 100 cc. of concentrated ammonium hydroxide. Shake well and keep at 50-70° for 8 hours with occasional shaking. Let stand over night at room temperature. Warm again to 50-70° and gradually dilute to 1 liter with water, not permitting the temperature to fall below 50° until dilution is complete. The resulting solution is red with a yellow tinge. Add excess of concentrated hydrochloric acid and boil a few minutes. Roseo-cobaltic chloride,  $[\text{Co}(\text{NH}_3)_5\text{H}_2\text{O}]\text{Cl}_3$ , is precipitated. The solution may be cooled in running water to hasten precipitation. Filter, wash on the filter with 1 per cent hydrochloric acid, and dry in an oven at 100°. For use dissolve 2.7 grams in about 400 cc. of water and 100 cc. of concentrated ammonium hydroxide and dilute to 1 liter.

*Chromate.* Dissolve 0.420 grams of ammonium bichromate in about 500 cc. of water. Add 100 cc. of concentrated ammonium hydroxide and dilute to 1 liter.

*Copper sulfate.* Dissolve 2.49 grams in 500 cc. of water, add 100 cc. of concentrated ammonium hydroxide and dilute to 1 liter.

The cobalt and chromium solutions are stable for at least 1 year. The copper solution precipitates in a few weeks unless it is hermetically sealed.

The 88 possible blends obtainable with various mixtures totaling 12 cc. of this series have been recorded <sup>11</sup> in Lovibond units. These consist of 36 spectrum colors in which only 2 solutions have been used, 27 hues in which only 1 cc. of a third solution is used, 18 blends in which 2 cc. of a third solution is used, 9 blends which are nearly grey and 1 grey containing 4 cc. of each solution. In order to record some of the less brilliant samples, negative values of Lovibond glasses had to be used. This means that the color glass was added to the standard to obtain a match, rather than to the sample.

**Permanganate-Chromate Series**—For this series neutral solutions of 0.001 *N* potassium permanganate and 0.01 *N* potassium bichromate are used. They match purples unobtainable from the previous two standards. They are not stable if exposed to organic contamination, and in any case must be used within 1–2 hours.

**Standard Mixtures**—Some typical mixtures follow, expressed in parts by volume.<sup>12</sup>

*Cobalt-Iron-Copper Series*

	Cobalt	Iron	Copper	Water
Standard caramel .....	4	7	1	0
Nessler nitrogen 1:500,000.....	3	9	0	12
Nitrate by phenolsulfonic acid method 1:500,000.....	0	12	0	6

*Cobalt-Chromate-Copper Series*

	Cobalt	Chromate	Copper	Water
Phosphoric acid by the molybdate method 1:20,000.....	0	12	0	68
Vanillin by Folin's method 1:100,000.....	3	3	10	0
Uric acid by Riegler's method 1:40,000.....	2	2	8	0
Salicylic acid by the ferric chloride method 1:50,000.....	7	1	5	6

<sup>11</sup> H. V. Army, *J. Am. Pharm. Assoc.* 2, 76-80 (1913).

<sup>12</sup> H. V. Army and C. H. Ring, *J. Am. Pharm. Assoc.* 4, 1294-9 (1915).

*Chromate Permanganate Series*

	Chromate	Permanganate	Water
Nitrite 1:10,000,000.....	15	1	13

By blending 10 volumes of 0.0025 *N* permanganate and 4 volumes of acid copper standard a reddish blue match for tincture of cudbar was obtained, stable for one-half hour. Comparisons were in a 0.5 inch Lovibond cell.

**Comparison of Standards**—Mixtures of 4 parts of the three colors, of either the acid or ammoniacal series, give a neutral grey, showing that apparently these metals give equivalent intensities of different colors at the same ionic concentration. That this does not hold for all metals is well known.

The use of ordinary "C. P." chemicals gives results indistinguishable from those with exceptionally pure chemicals. Similar neutral standards precipitate within a few days. Acid standards prepared in 1914 and restandardized in 1919 had the same values. A review<sup>13</sup> has been published, and trouble with iron and cobalt chlorides reported.<sup>14</sup>

The use of a methyl orange solution as a stable standard in determination of titanium has been proposed.<sup>15</sup> For beer and worts<sup>16</sup> cobalt and bichromate mixture is superior to ferric alum, iodine or aniline dye solutions.

**Errors**—Artificial standards are open to the following objections:

1. If an unstable substance is being determined, a standard of the same material will decompose at approximately the same rate, while an artificial standard does not decompose. The result is a longer period during which correct results can be obtained with the natural standard than with the artificial.

2. The artificial standard must be calibrated in terms of the natural standard. The sample must then be read in terms of the artificial standard. A double possibility of error is introduced.

3. The artificial standard, while apparently the same in color, is not actually the same in wave length distribution.<sup>17</sup> Individuals vary in their

1. G. Mellon, *Proc. Indiana Acad. Sci.* 1922, 164-71.

C. Whipple, *Eng. Contr.* 62, 80-4 (1924).

<sup>15</sup> A. Gautier, *Rev. gén. chim.* 14, 16 (1911).

3. Lampe, *Woch. Brau.* 39, 235-6 (1922).

<sup>17</sup> J. P. Mehlig and M. G. Mellon, *J. Phys. Chem.* 35, 3397 (1931).

sensitiveness to different colors.<sup>18</sup> If the components of the two colors are different, as will always occur with artificial standards, irregular results will occur as the operators differ in sensitiveness to the colors present. This error can also occur with natural standards in matching different concentrations when Beer's law does not hold exactly.

Comparison of bichromate standards<sup>18</sup> of the same concentration at the same height in the colorimeter against sodium picramate gave readings inversely proportional to the concentration of the latter. When the height or concentration of the bichromate solutions was not the same, the readings were not always proportional. This is a case where the use of the bichromate as artificial standard depends on careful standardization at a definite concentration of bichromate and setting of the instrument. An equation to represent the relation between picramic acid and picric acid was developed. By addition of a buffer to chromate solutions the equilibrium between chromate and bichromate can be fixed by fixing the pH value. A definite reproducible color would then be obtained.<sup>20</sup>

The suggestion has been made that stable dye solutions would be suitable in many cases.<sup>21</sup> Exposure tests on 86 dyestuffs in glycerol showed fading in sunlight by all but 3 within 2 weeks. Two of those 3 faded within 2 months.<sup>22</sup> Many inorganic salts also fade in glycerol. In water, dyestuffs are more stable, 13 out of 109 being unchanged after 3 weeks in the sunlight. Similar comparison of 35 naturally colored liquids showed fading in 2 weeks or less in all cases. Many inorganic salt solutions in water also fade. The most stable are acid ferric chloride, uranyl chloride, cobalt chloride, cobalt sulfate, cupric chloride, cupric sulfate, sodium bichromate, sodium chromate, nickel chloride and nickel sulfate. In many cases precipitates form on standing. The color of iron salts is greatly altered by change of temperature.

A caramel standard for carbon in steel has been used<sup>23</sup> but is particularly objectionable because of the great variation between various lots of caramel, even when made under closely standardized conditions. The iron-cobalt-copper series of acid standards is much less objectionable for this purpose.<sup>24,25</sup>

18 G. Falk and H. M. Noyes, *J. Biol. Chem.* 42, 109-30 (1920).

20 Holger Jorgensen, *Biochem. Z.* 186, 485-9 (1927).

21 H. Eckstein, *Chem.-Ztg.* 52, 317 (1928).

22 J. E. Doherty and J. F. Ahearn, *Oil and Soap* 11, 46-52 (1934).

23 H. LeChatelier and F. Bogitch, *Ann. chim. anal.* 22, 193-225 (1917).

24 J. Blodget Britton, *Chem. News* 22, 101 (1870).

25 Eggertz, *Chem. News* 44, 173 (1881).

Some of the Clark and Lubs pH indicators have been matched by permanent standards.<sup>26</sup> Mixtures of 2 cc. of 20 per cent cobalt nitrate and 98 cc. of 0.03 per cent potassium bichromate, and of 5 cc. of 20 per cent cobalt nitrate and 95 cc. of 10 per cent copper sulfate correspond to pH 6.0 and pH 7.6 respectively with bromothymol blue. Graded mixtures give the intermediate values. By use of 4 cc. of 10 per cent cobalt chloride and 96 cc. of 0.03 per cent potassium bichromate, and of 26 cc. of 10 per cent cobalt chloride and 74 cc. of glacial acetic acid in differing proportions methyl red and phenol red respectively were duplicated. Much other work has been done on artificial standards for pH which cannot be given in detail.<sup>27-34</sup>

For high concentrations of picramate and low concentrations of picrate 1 mg. of the former may equal as low as 200 mg. of the latter, whereas in the opposite concentrations 1 mg. of picramate may equal more than 1500 mg. of picrate. Intermediate values are of the order of 1:1000. Uncertainty is thus produced in correcting for excess picrate in solution and accuracy is possible only at predetermined concentrations. In an extended investigation the only artificial standard recommended<sup>34</sup> as optically satisfactory was potassium chromate or bichromate for matching monochromatic indicators such as *m*- or *p*-nitrophenol and  $\alpha$ - or  $\gamma$ -dinitrophenol.

Commercial grey standards<sup>37</sup> have been criticized<sup>38</sup> because of a 10 per cent decrease in absorption in 15 days in daylight, with particular deterioration of the red. They have been defended as satisfactory,<sup>39</sup> one in particular<sup>39a</sup> being stable for a year if stored in a cool, dark place.

Regardless of the above weaknesses there are many determinations in which artificial standards must be used. For creatinine, potassium bichromate was used for many years because the natural standard was not

<sup>26</sup> P. Bruere, *J. pharm. chim.* [8] 3, 377-9 (1926); 4, 241-7 (1926).

<sup>27</sup> H. D. Haskins, *J. Lab. Clin. Med.* 4, 363 (1919).

<sup>28</sup> I. M. Kolthoff, *Pharm. Weekblad* 59, 104, 129 (1922).

<sup>29</sup> C. Risch, *Biochem. Z.* 148, 147 (1924).

<sup>30</sup> A. Janke and S. Kropacsy, *Biochem. Z.* 174, 120 (1926).

<sup>31</sup> P. Bruere, *J. pharm. chim.* [8], 3, 377-9; 4, 241-7 (1926).

<sup>32</sup> A. Taub, *J. Am. Pharm. Assoc.* 16, 116 (1927).

<sup>33</sup> H. Jorgensen, *Biochem. Z.* 186, 485 (1927).

<sup>34</sup> J. P. Mehlig and M. G. Mellon, *J. Phys. Chem.* 35, 3397-414 (1931).

<sup>37</sup> A. Thiel, *Z. Elektrochem.* 38, 621-2 (1932).

<sup>38</sup> E. Landt, *Z. Elektrochem.* 39, 310-12 (1933).

<sup>39</sup> A. Thiel, *Z. Elektrochem.* 39, 312-16 (1933).

<sup>39a</sup> A. Thiel, *Chem. Fabrik* 1934, 383-4.

available. This practice has been rendered unnecessary by the commercial production of creatinine at a reasonable price.<sup>40</sup>

**Systems of Color Recording**—There are three which will not be discussed in detail, the Munsell System,<sup>41-43</sup> the Ridgeway System of 1115 colors<sup>44</sup> intended for ornithologists, and the Ostwald System of 2500 colors<sup>45</sup> abridged to 768 colors.<sup>46</sup>

---

<sup>40</sup> E. Guy Hopkins, R. F. McCracken and W. F. Sharpe, *Bull. Med. Coll. Va.* 23, No. 3, 24-6 (1926).

<sup>41</sup> A. A. Munsell, "A Color Notation," Boston (1907).

Munsell Color Co., "Atlas of Munsell Color System," New York.

I. C. Priest K. S. Gibson and H. J. McNicholas, *Technological Paper, Bur. of* s No. 167 (1920).

Ridgeway, "Color Standards and Color Nomenclature," Washington, D. C. (1912).

<sup>45</sup> W. Ostwald, "Der Farbenatlas," Leipzig (1918).

<sup>46</sup> W. Ostwald, "Der Farbenatlas," Leipzig (1919); *Z. angew. Chem.* 42, 437-9 (1929).

## CHAPTER VII

### ACCURACY

OF THE factors limiting the accuracy of colorimetric methods, some are inherent in the nature of the comparison to be made and some in the specific procedure of a given determination. The limitations are much fewer for the Series of Standards and Duplication Methods than for the Dilution Method and Balancing Method.

Colorimetric methods may be classified according to the reason for their use. Some find their popularity because they are rapid. Accuracy is sacrificed for speed in obtaining the final result. A second class is used because it furnishes a method of determination of small amounts of substances with greater accuracy than is possible by gravimetric or volumetric methods. A third class contains methods where no gravimetric or volumetric method is available. Biological methods in particular are apt to be of this third class. The determinations of the first class may all be carried out in a short time. It often happens that the method of preparation of the sample for the second and third classes takes hours to insure the accuracy desired. That they are in many cases more accurate for estimation of small quantities than the majority of volumetric or gravimetric methods is indicated by their use in specifications for the purity of chemicals.<sup>1</sup>

**General Limitations**—For a colorimetric method to be accurate the color produced by the action of the given reagent on the test substance should be the only color present in the solution. Therefore a colorimetric estimation is not ordinarily possible if the original solution is colored, unless that color is produced by the test substance or will be removed by the reagent used. In some particular instances a slight contamination of one color may be equalized by coloring the standard. In a few types of determinations the color of the original solution is corrected by a similar colored layer in addition to the standard. Similarly a colorimetric estimation is not possible if the solution to be examined contains anything

---

<sup>1</sup> W. D. Collins, H. V. Farr, Joseph Rosin, G. C. Spencer and Edward Wickers, *Ind. Eng. Chem.* 18, 636-9 (1926) et seq.

other than the test substance which will give a precipitate or color with the given reagent. It is desirable for the accuracy of these methods that the color produced be reasonably permanent and that the conditions under which it was produced be such that they can be duplicated without great difficulty. In an exceptional case comparisons of colors permanent for only 30 seconds have been successfully carried out.

**Limitations of Specific Methods**—Each of the four types of methods is particularly applicable to certain special conditions, and not applicable under other conditions.

The *Series of Standards Method* is the most widely applicable. If the color developed from the test substance fades, it can be estimated by comparison with a series of standards prepared at the same time under the same conditions. This method does not assume that Beer's law holds.

The *Dilution Method* is based on the assumption of Beer's law. If the color is not proportional to the amount of test substance over a considerable range this method cannot be used. Permanence for only a short time is necessary.

The *Balancing Method* depends on use of a variable thickness of layer instead of addition of water as in the dilution method. Beer's law is therefore assumed to hold. Ordinarily the color developed must be permanent.

The *Duplication Method* is subject to one very important limitation, the color must develop at once and be permanent for a few minutes. It does not assume that Beer's law holds. If the color developed varies with the conditions under which mixing is carried out, this method cannot be used.

**Typical Sources of Error**<sup>2</sup>—In Folin's creatinine estimation various sources of error have been suggested. In a generalized form these may be expressed in the following list.

1. Mechanical errors of the colorimeter.
2. Optical errors of the solutions.
3. Errors from varied light.
4. Errors in readings.
5. Errors of dilution.
6. Errors from varied temperatures.
7. Errors from varied times of standing.

<sup>2</sup> William M. Dehn, *J. Am. Chem. Soc.* 39, 1392-9 (1917).



8. Errors from variable quantities of chemicals present.
9. Errors from other solutes than the test substance present in the sample.
10. Unavoidable error of the individual operator.

To these might be added selection of an improper range of concentration,<sup>3</sup> variable size of colloidal particles, dichromatism, turbidity, impurities in the reagents and errors in artificial standards. Adsorption on the glass, particularly by aniline dyes has been reported as a source of error.<sup>4</sup>

**Mechanical Errors of the Colorimeter**—Errors due to inaccurate calibration can always be checked by comparison of two known solutions. This should be done with a new colorimeter before putting it into use, but the range of accuracy of the solutions used must be considered.

Be sure that cups are not interchanged due to possible variations in thickness of the bottoms. Dust in the eyepiece or stains on the cups will cause errors. Reflectors and color screens must likewise be free from dust or other deposits.

A case has been reported<sup>5</sup> in which one plunger of a Dubosecq instrument had dropped about 3 mm. and the other 5-6 mm. due to softening of the wax with which they were held in place. Similar results with other instruments were stated to occur. Even if both had dropped the same distance the results would be in error. This is avoided by checking the zero point of the instrument before use.

**Optical Errors of the Solutions**—These may arise from color in the solution other than that of the test substance. Methods are usually so chosen as to prevent such errors. Other and more important errors are inherent in the design of instruments.<sup>2</sup> In order that colors which appear identical may actually be so, they must be viewed at equal distances, and the lines of the major axes of the two must intersect at a point slightly behind the center of the retina. This is impossible of attainment, as optically a cylinder appears as a truncated cone. The condition is approached in practice by having the cylinders at a considerable distance from the eye. When an instrument is used for comparison the condition is further approached by close juxtaposition of portions of the centers of the fields at substantially equal distances from the eyepiece. If depths

<sup>3</sup> N. E. Pestov, *Z. anal. Chem.* 89, 9-17 (1932).

<sup>4</sup> N. Roberts, *Bull. Hyg. Lab. P. H. and M. H. Serv.* 66, (1910).

<sup>5</sup> J. H. Long, *J. Am. Chem. Soc.* 38, 716 (1916).

and cross sections are identical and observed at equal and reasonable distances from the eye the errors of optical distribution may be ignored.

When the cross section varies but the depth is the same, by viewing a limited area in the center of standard and sample the errors of cross section do not appear. When the depths of the two solutions vary, as in the case of other errors may arise. It is conceivable that the errors may vary according to the same law that

governs optical size of objects, inversely as the square of the distance from the point of vision. This can neither be proved nor disproved hence the assumption that different columns of liquids of the same apparent tint contain the same number of molecules is made for balancing and dilution methods.

In equal colors of unequal depth more molecules will be behind each other in the longer column than in the shorter. This error is ignored and is a possible source of error in comparing a standard with a sample of widely varying concentration. It is minimized in practice by having the concentrations of the two reasonably close.

The proper shape of container for the solution is a section of a hollow sphere with the point of vision coinciding with the center. In comparing fairly long columns this condition is approximated. In comparing shallow solutions the error becomes greater and an appreciable difference in color between the edge and center of the field can be detected.

In case of considerable variation in concentration Dehn<sup>2</sup> has shown the following error:

$a$  = distance for nearer surface.

$a + b$  = distance from further surface.

$r$  = radius of nearer surface.

$c$  = distance radius of nearer surface is moved toward the further surface.

The ratio of the viewed volumes is assumed to be

$$1 : b - c/b \quad (I)$$

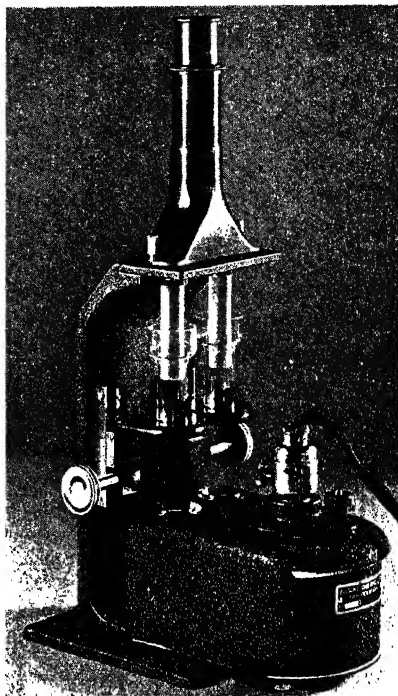
The actual ratio is

$$1 : \frac{(a + c)^2 (a + b)^3 - a^3}{(a + c)^2 (a + b)^3 - a^3} \quad (II)$$

If  $a = 2$ ,  $b = 2$  and  $c = 1$  the ratio according to equation I is 1 : 0.500 and according to equation II is 1 : 0.290, an error of 42 per cent. At  $a = 20$ ,  $b = 2$ ,  $c = 1$  the ratio according to I is 1 : 0.475 or equal to the assumed limitation of the colorimeter.

**Errors from Varied Light**—If two colors being compared are not alike in quality the results with varied light differ greatly. Comparison of such solutions is not satisfactory at best. Considering possible qualities of light<sup>8</sup> such as light from a blue sky, the brassy sky of summer, high white light, fog light, dark cloud light and the dull light of rainy days it is not surprising that individual estimations may vary with the light. Taylor found that on some days of low fog in California he could not estimate creatinine closer than  $\pm 10$  per cent, while under the best conditions he could estimate it to 1 per cent. This was using an artificial standard of bichromate.

Diffused daylight is free from most of the disadvantages of direct light.<sup>9</sup> By standardizing the intensity of light, angle of incidence, degree of diffusion, distance between the eye and the object, and time of making the observation, constancy of observations can be obtained. It is to be noted that three of the five factors listed refer to light. These are also readily standardized by use of some source of artificial light. In the comparison of six water-soluble colors corresponding to the six spectrum colors, color readings were constant between 16 and 26 units. Below 16 the readings vary, each color having an idiosyncrasy of its own. Readings are uniform with light varying from a  $40^\circ$  angle to perpendicular. Between  $40^\circ$  and  $70^\circ$  the variation is peculiar to the color. Light of 18 to 28 units from a north window is best. Passing clouds may change the reflection of direct rays.



Colorimeter Lamp for Attachment Directly to Colorimeter. (Courtesy of Bausch & Lomb Optical Co.)

<sup>8</sup> Alonzo Englebert Taylor, *J. Biol. Chem.* 9, 19 (1911).

<sup>9</sup> J. W. Lovibond, *J. Soc. Chem. Ind.* 28, 500-3 (1909).

The color of a solution may be nearly pure but is much more apt to be a combination of two or more distinct bands. In that case the color of the solution may vary with the depth observed and the concentration of the colored test substance. A solution of bichromate is an example; in dilute solution it is apparently a pure yellow, in more concentrated solution red lines are also present. Such cases are well recognized among pH indicators. In the same way turbid solutions frequently show quite different colors if the colored test substance is dichromatic.

This dichromatism may be intensified or lessened by the use of artificial illuminants which are in general rich in red waves and deficient in blue. Thus a dichromatic indicator will be redder in such artificial light and less blue. In many cases errors due to dichromatism are avoided by use of colored light for the comparison, the interfering color being absent from the colored light used. As an example blue or yellow solutions may be compared in the light of a mercury arc to eliminate trouble due to dichromatism in the red waves. The problem of standard lighting is best answered by some source of standard, artificial light. Many types of colorimeters include artificial lighting as an integral part of the instrument.<sup>10</sup>

The light of the sun that reaches us is more yellow than the original, due to some absorption of blue by the atmosphere. Blue skylight is the opposite extreme, with light reflected from white clouds intermediate. The evening sky and the light on misty days and in the city is often more yellow than direct sunlight. It is feasible to remove some of the red end of the spectrum from artificial light to duplicate sunlight but not to add blue light to make up the deficiency.<sup>11</sup>

It is customary to adopt as a standard white light for computation purposes the energy distribution of a black body at 5000° K. Such a light source is not obtainable in the laboratory. Practically, a source of much lower temperature is used with a bluish filter. Copper and cobalt sulfate filters for this purpose have been described<sup>12</sup> for use with a lamp operated at 2360° K to maintain it as a substandard of 3000° K.

Many practical forms of lighting equipment are available. A light in a box, painted white inside and provided with a slit by which the light can illuminate the mirror of the colorimeter is satisfactory.<sup>13</sup> The color committee of the American Oil Chemists' Society used as a standard

<sup>10</sup> J. H. Killan, *J. Lab. Clin. Med.* 10, 570-3 (1925).

<sup>11</sup> F. E. Lamplough, *J. Soc. Dyers and Colourists* 38, 265-9 (1923).

<sup>12</sup> J. Guild, *Trans. Opt. Soc.* 27, 124 (1925-6).

<sup>13</sup> H. D. Kay, *Proc. Physiol. Soc., J. Physiol.* 62, 31 (1927).

light such a box containing a 150 watt Mazda lamp whose light was reflected from a magnesia block.<sup>14</sup>

**Errors in Readings**—Nearly all methods in the range for which they are designed claim an accuracy of 5 per cent or better. It has been stated<sup>15</sup> that the eye cannot perceive differences of less than 5 per cent. While true for a series of standards such as were used by Arny and Ring, this is not generally true. Results to 1.2 per cent have also been estimated<sup>16,17</sup> and high accuracy due to concordant results claimed.<sup>18</sup> If an instrumental method is used 2 per cent is probably obtainable from several readings with the majority of methods, in their range of greatest accuracy, and 1 per cent with many methods.

One method of obtaining improved accuracy is to make the comparison of sample and standard at several different dilutions.<sup>19</sup> The accuracy of an estimation can also be increased by comparing the developed sample with 2 distinctly different intensities of color rather than with a single color intensity.<sup>20</sup> The range of optimum accuracy is somewhat altered by factors such as the nature of the light, the observer, etc.

Tabulation of the accuracy of 4000 readings as affected by eye adaptability and fatigue shows that a green color read with both eyes is most accurate.<sup>21</sup> This work included experiments with 14 persons, the use of blue, green, yellow and red solutions, and light from four sources. Comparisons were made with each eye alone and with both eyes. The range of optimum accuracy of any individual eye can be accurately measured.<sup>22</sup>

When the accuracy of readings is in question, usually due to variations from Beer's law and to individual error, a correction curve should be constructed. To do this, take a known solution of the test substance to be used. Prepare a series of dilutions such as 2.0, 1.8, 1.6, 1.4, 1.2, 0.8, 0.6, and 0.4 times the strength to be adopted as a reference standard. This reference standard is conveniently half that of the original solution. Adopt a height of reference standard similar to that of the unknowns to

<sup>14</sup> David Wesson, *Cotton Oil Press* 6, 36 (1922).

<sup>15</sup> H. V. Arny and C. H. Ring, *J. Am. Phar. Assoc.* 4, 1294-9 (1915); *J. Ind. Eng. Chem.* 8, 309-17 (1916).

<sup>16</sup> P. Fleury, *Bull. soc. chim. biol.* 4, 223-32 (1922).

<sup>17</sup> Charles W. Folkard, *Chem. News* 75, 73 (1897).

<sup>18</sup> Y. Garreau, *Bull. soc. chim. biol.* 4, 233-4 (1922).

<sup>19</sup> Rudolf Klockmann, *Z. angew. Chem.* 43, 993-5 (1930).

<sup>20</sup> Frederick L. Hahn, *Z. anal. Chem.* 90, 330-2 (1932).

<sup>21</sup> C. Frick, *Chem. Fabrik* 1932, 481-4.

<sup>22</sup> J. Guild, *Phil. Trans. Roy. Soc. (London)* 230, 149-87 (1931).

be tested and compare it against each of the diluted standards and a portion of the reference standard. The values obtained can then be plotted in comparison with the theoretical straight line curve, and deviations determined. Similar curves for 10, 15 and 20 mm. depths of reference standard are desirable. Provided these do not coincide with the reference standard, and they usually do over a fair range, the values can be correctly read from the curves so determined rather than taken on the assumption that Beer's law holds. The alternative and preferable procedure is to discard all results outside of the range in which Beer's law is so shown to hold experimentally. The deviations from Beer's law will ordinarily follow the same formula as do nephelometric corrections.

Published curves for these values should not be used as they fail to account for factors which are difficult to define but which are probably inherent in the individual operator and his instrument.

In attempting to attain a high degree of accuracy by colorimetric methods, due account must be taken of other errors inherent in the methods. Accuracy to 0.1 per cent in such calibration curves is hardly justifiable if the concentration of unknown and the nature of the method prohibit accurate reading to closer than 5 per cent. Such deviations will be apparent in the construction of the correction curves. Construction of such curves is a highly desirable experience for the student or the analyst lacking experience with colorimetric methods.

It is desirable to avoid methods by which factors for formulae are worked out and used, as there is a tendency to over-extend the use of such formulae, a thing improbable when a visual comparison of the curves must be made. Substitution in Kober's formula  $K = x(xy - s)/s(x - 1)$  permits comparison of curves obtained at different levels by the same worker or different workers. In this formula  $y$  is the reading of the solution varied,  $x$  the ratio of the concentrations of the known and unknown and  $s$  the setting for the solution which is not varied. This leaves only  $K$ , a constant for the solution being compared provided the values fall on a straight line.

**Errors of Dilution**—In many cases it is impossible to choose a sample having as small a content of the test substance as specified in the method, particularly if the substance being analysed is an ore or alloy containing a fairly large percentage of the test substance. In that case the sample may be taken containing more of the test substance than specified for the method and dissolved as directed. This solution may then be accurately diluted and an aliquot taken. If the method specifies that a sample must

contain less than 0.001 gram of the test substance and the smallest sample of the substance which can conveniently be weighed out will contain nearly 0.1 gram of test substance, this may be dissolved as specified and diluted to one liter in a volumetric flask. Ten cc. of this may then be treated as directed. The weight of test substance found in that amount of solution multiplied by 100 will give the amount in the original sample.

When it is necessary to use such a method the final result cannot be as accurate in regard to the absolute amount of test substance by weight, but the results are as accurate so far as the percentage error is concerned. As an illustration, accuracy to 5 per cent with a weight of sample containing 0.001 gram of test substance would permit an error of 0.00005 gram of the test substance. If the sample contained 0.5 gram of test substance, an amount greater than would ordinarily be present in a weight of sample used, the corresponding accuracy to 5 per cent would permit an error of 0.025 gram. In the second case the amount of test substance in the sample is 500 times as large and the possible error in grams 500 times as great. Since comparison is by the same method, the larger sample being diluted for the purpose, the percentage error is the same in each.

The rapidity with which colorimetric readings may be made often admits of the attainment of considerably greater accuracy than estimated, by rapidly taking several readings and averaging the results obtained.

Some colors change not only in intensity but in quality at different dilutions.<sup>23</sup> This is not usual with natural standards but occurs with Nessler's reagent.<sup>24</sup> With artificial standards it is frequently the case. Many of these errors<sup>25</sup> with natural standards are due to two colors being present. In such cases one may change at a different rate from the other on dilution; that is, one may not follow Beer's law.

It is always desirable by the balancing method to compare a sample with a standard having a content of test substance varying from that of the sample by not over 50 per cent. In many cases the difference must be less. Gross errors from this source in the determination of creatine and creatinine have been referred to. In many cases several standards are available and a proper one can be selected. This depends on stability of the color developed, time required for preparation of standard, and effect of dilution on sample or standard. The only general rule which can

<sup>23</sup> M. Lukiesh, "Color and Its Applications," 2nd ed., p. 23 et seq., D. Van Nostrand Co., New York (1921).

<sup>24</sup> D. W. Horn, *Am. Chem. J.* 35, 253-8 (1906).

<sup>25</sup> K. George Falk and Helen M. Noyes, *J. Biol. Chem.* 42, 109-30 (1920).

be stated is that the accuracy of the balancing method will be greater if the standard does not differ greatly in concentration from that of the sample.

In some cases the solute molecule itself may be ionized and the color measured is that of one of the ions. In such a case the assumption made, is that ionization is complete and that all of the test substance in standard and sample is being measured. In general this is correct. More commonly the solute molecule is little, if at all, ionized.

**Errors from Varied Temperatures**—In many determinations the sample and standard are heated to develop the color. In such cases the color may vary with temperature. If comparison is made with a standard which has been standing, the temperature of standard and sample may not be the same and may result in error. In some cases a change in intensity or quality of color with temperature has been noted in the outline of the method.

**Errors from Varied Times of Standing**—In the same way in many determinations the color develops only after standing for a definite period. If the comparison is made before sufficient time has expired, erroneous estimations are obtained. Such reactions are usually a matter of temperature, so that standing for five minutes in a room at 15° may not give at all the result of 5 minutes at 25°

Another error occurs with solutions that fade. If a solution stands too long the intensity may have decreased. An extreme case of these errors has been described by Philip B. Hawk in the determination of vitamin A.<sup>26</sup> This type of error may be avoided by preparation of the same quantity of sample and standard at the same time.

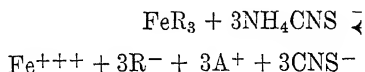
**Errors from Variable Quantities of Chemicals Present**—In many cases the color is influenced by the amount of chemicals added in the preparation of sample or in the development of color. If the standard does not contain the same amounts of neutral salts as the sample, this may lead to error. In the development of color the concentration of reagent should not differ in sample and standard. In many cases the mass action effect of a large excess of reagent will tend to darken the color. An example of this is one of the oldest colorimetric methods, that for ferric iron with thiocyanate. The general chemistry experiment whereby the intensity of red color is increased by addition of more thiocyanate, or

---

<sup>26</sup> See Vol. 2.



lessened by addition of ammonium salts, is clearly a mass action effect. The reaction is expressed in two forms by the following equations.



It has been shown<sup>27</sup> that  $(\text{NH}_4)_9\text{Fe}(\text{CNS})_{12}$  does not exist. The most recent indication<sup>28</sup> is that the color in aqueous solution is due to a  $[\text{Fe}(\text{CNS})_6]^\equiv$  ion and in ether solution to  $\text{Fe}[\text{Fe}(\text{CNS})_6]$ .

A study made on picric acid solutions illustrates the effect of variation in the solvent, which may be considered a special case of this type of error.<sup>29</sup> Up to 0.007 per cent solutions, in absolute alcohol, 95 per cent alcohol, water, sodium hydroxide and ammonium hydroxide all had the same intensity of color. In concentrated solutions the color ratios varied in the order 1, 3.1, 10, 22.5, 24. This is explained by different equilibria between the quinoid and benzoid forms and by different degrees of hydration of the molecules. The ratios of the colors in the solutions are also affected by temperature.

For many reactions the hydrogen-ion concentration must be limited to a definite range for the results to be comparable. The effect of acetic acid and of hydrochloric acid on the color of methyl orange varies greatly at the same pH. Acetic acid gives the stronger color up to  $1 \times 10^{-3}$  moles per liter while above that concentration, hydrochloric acid gives the deeper color. Non-electrolytes do not effect the sensitiveness but neutral salts increase it, due to change in the degree of dissociation of the methyl orange.

These errors are avoided by careful standardization of the method. In some cases the effect of variable concentration of reagent, or of hydrolysis due to variable concentration of test substance, is to produce dichromatic or polychromatic changes, in which the standard and sample are not comparable at all. Such cases can easily be shown by the Ives tint-photometer. Horn<sup>30</sup> has demonstrated this effect of concentration of test substance, as has also Dehn.<sup>29</sup>

**Errors from Other Solutes than the Test Substance Present in the Sample**—If the reaction used is a general rather than a specific

<sup>27</sup> K. Jablczynski and W. Stankiewicz, *Koczniki Chem.* 7, 549-58 (1927).

<sup>28</sup> H. I. Schlesinger and H. B. Van Valkenburgh, *J. Am. Chem. Soc.* 53, 1212-16 (1931).

<sup>29</sup> William M. Dehn and Alice A. Ball, *J. Am. Chem. Soc.* 39, 1381-92 (1917).

<sup>30</sup> D. W. Horn, *J. Am. Chem. Soc.* 36, 329 (1914).

one, the result obtained may be greatly in error. This is avoided as far as possible by naming the interfering substances. Cresol gives the same reaction with phosphotungstate as phenol. When both are present, neither can be accurately determined.

A possible source of error due to interfering ions has been pointed out by Thayer.<sup>32</sup> Phosphorus, silica and arsenic all give the same type of yellow color with molybdate reagent. Therefore if arsenic is present, phosphorus cannot be determined by the molybdate method. The effect of iron alone or of phosphate alone on molybdate solutions used for estimation of silica, is entirely different from the combined influence of the two, and from the effect of iron in the presence of silica. Broad statements that certain ions do not interfere because they do not react with the reagent, are therefore unjustified, unless it is known that they also do not react with each other, or with the test substance to form compounds which do react with the reagent.

In many cases of colloidal dispersion of test substance the size of aggregate will be affected by the kind and concentration of dissolved salts. An example is colloidal lead sulfide for the determination of lead. This method although widely used is often unsatisfactory, giving yellow to brown colors in many cases, which cannot be matched, because of variation in the "salt effect" of dissolved substances. To some extent this effect is overcome by addition of a protective colloid. The equal concentration of all chemicals in sample and standard is as important with colloidal sols as with true solutes.

Interference due to the natural color of a solution may be corrected by similarly coloring the standard comparison solution.<sup>33</sup> A more satisfactory method is to superimpose the colored solution over the standard.<sup>34</sup> This permits using a portion of the sample itself and is correspondingly more accurate. This principle is applied in the block comparator.<sup>35</sup>

A somewhat more involved method of avoiding interfering colors is as follows.<sup>36</sup> Develop the color in a fairly concentrated pure solution of the substance to be determined. Pour the colored solution into a container having flat sides, and obtain its absorption spectrum with a simple hand spectroscope, using the same source of light as that to be used with the colorimeter. Dilute the solution and repeat the examination. This will

<sup>32</sup> Lewis A. Thayer, *Ind. Eng. Chem., Anal. Ed.* 2, 276-83 (1930).

<sup>33</sup> S. P. L. Sorensen, *Compt. rend. trav. Lab. Carlsberg* 8, 396 (1909).

<sup>34</sup> G. S. Walpole, *Biochem. J.* 5, 207 (1910).

<sup>35</sup> See p. 11.

R. H. Hamilton, Jr., *Science* 75, 563-4 (1932).

indicate the region where the maximum change in absorption of the color developed by the reagent occurs. Examine in the same way glass or gelatine filters, or solution of chemicals having the desired complementary color, in order to find one which transmits light in the region of maximum absorption by the above solution, but which absorbs nearly all other light. Superposition of the concentrated solution in which the color has been developed, and of the filter will additively absorb nearly all visible rays. Place the selected glass filter over the eyepiece of the colorimeter. If a solution is used as filter, place it in a flat-sided jar in front of the colorimeter so that the light is modified before it reaches the reflecting mirror. The transmission of the interfering color will thus be prevented.

In the last analysis, errors from this source may arise which are difficult to detect. If the test substance is one which can be isolated in a sufficient state of purity, comparing known amounts in solutions which contain possible interfering substances which may occur in the sample solution, demonstrates the interference or noninterference of the latter. Some methods, mainly biological, are used as a matter of necessity without the effect of accompanying substances being known. Where substances are known to interfere, their effects are quoted with the method.

**Unavoidable Error of the Individual Operator**—These errors are avoided by careful training of the operator and by checking of his accuracy by other operators. Avoiding eye fatigue due to long continued operation is essential to accuracy.<sup>37</sup> The best time of observation is about 3 seconds for a dark color or 5 seconds for a light color.<sup>38</sup> To avoid eye fatigue the intensity of illumination should not be greater than is necessary to make observations. The eye not in use should not be closed and a wearer of spectacles should not remove them, unless other means are provided for the correction of the eye.<sup>39</sup>

Color blindness does not necessarily bar a man from colorimetry, as it usually exists for 1 or 2 tones only, and gives the man more than ordinary sensitiveness to shade.<sup>40</sup> Some color is usually present in the range to which the eye is sensitive. Consequently changes are detected and, provided the transmission of the sample and standard are the same, accuracy may be expected to be limited only to the extent that the intensities being measured are less than the normal. Gross errors may

<sup>37</sup> N. E. Pestov, *Z. anal. Chem.* 89, 9-17 (1932).

<sup>38</sup> J. W. Lovibond, *J. Soc. Chem. Ind.* 28, 500-3 (1909).

<sup>39</sup> Am. Soc. Testing Materials, Standards 1930, 905.

<sup>40</sup> N. Roberts, *Bull. Hyg. Lab. P. H. and M. H. Serv.* 66, 3 (1910).

easily occur with artificial standards or in any case where standard and sample do not have the same form of transmission curve. Color ignorance on the part of new workers, while important in classification of colors, does not affect their value in colorimetry. The photoelectric colorimeter has been recommended for elimination of the personal factor.<sup>41,42</sup>

All people not color blind are normal after training, differences between individuals being due to physical conditions or lack of training.<sup>38</sup>

**Variable Sensitivity of Range**—The assumption is frequently made that a colorimetric method is equally sensitive throughout its entire range. That this is probably incorrect is indicated by careful consideration. If a method will detect the difference between 0.001 and 0.0008 mg. of test substance it is highly improbable that it will detect the difference between 0.1000 and 0.0998 mg. On the other hand the difference between 0.1000 and 0.0800 mg. should be much greater than between 0.001 and 0.0008 mg. It therefore appears that the law as to sensitiveness, if there is one, must be complex.

This factor has been studied quantitatively for solutions in the usual colorimetric range. The sensitiveness of a colorimetric method has been defined as the reciprocal of the weight in milligrams of test substance which can just be differentiated with certainty. A more practical definition of sensitiveness, one more commonly used, is the difference expressed in weight, which can normally be detected over the range of concentration in which the method is applied. The personal equation may be disregarded. The ease of determination may be measured by the variation from the mean when two concentrations are compared, with and without intermediate shades to aid in the comparison. Both the sensitiveness and the ease of determination vary with the concentration.

For chromium <sup>44</sup> as chromate the maximum sensitiveness is between 0.004 *M* and 0.008 *M*. To detect the color at least 0.000013 gram per liter must be present. A change of 0.000010 gram could then be detected. This indicates an unexpected factor, that it is easier to distinguish a difference between two colors than between colorless and colored. The green of chromic chloride was best studied at about 0.003 *M*.

For copper sulfate the form of the curve of accuracy was about the same as for chromate, but the tinctorial power was only 3.3 per cent as

---

<sup>41</sup> L. Blin Desbleds, *Worlds Paper Trade Rev.* 92, 1658-62, 1704, 1706, 1748-54 (1929); *Paper Maker and Brit. Trade J.* 78, 464A-464H (1929).

<sup>42</sup> Rene Toussaint, *Chimie et Industrie* 21, 924-30 (1929).

<sup>44</sup> D. W. Horn, *Am. Chem. J.* 35, 253 (1906).

great.<sup>45</sup> Chromate could be satisfactorily determined from 0.004 *N* to 0.000007 *N* while the range for copper was 0.6 *N* to 0.0013 *N*. For copper, the amount that will produce a difference in color at different concentrations, that can just be distinguished, is not a constant weight, but a constant fraction of the total weight present. This does not hold when the solutions become too concentrated or too dilute. As long as the work is within the range of maximum sensitiveness, it does not matter what concentration the analyst selects. The absolute error will differ but the percentage error will be the same. This may be an application of Weber's law, "The increment in stimulus that produces a just perceptible difference in sensation is always the same fraction of the total stimulus."

The sensitiveness for copper and cuprammonium ions is about the same, which indicates that this is probably a constant for the individual element, independent of color.<sup>46</sup> With respect to the possible color ranges of ammoniacal copper sulfate solution, in concentrated solution, the fraction of the whole needed to detect a change in color is 5.5 per cent, in the intermediate range it is only 1 per cent, and in the dilute range it varies and becomes very large. This explains the variations in sensitiveness found by different investigators with the same reaction, and to some extent the large variations in sensitiveness between different methods, when the range has not been investigated in detail.

Solutions of dyes have also been found to show differences perceptible, according to a geometric series.<sup>47</sup> The least perceptible difference increases with concentration of the solution. The phenomenon of a range of maximum sensitiveness is not shown by these results. The proposal is made to calculate the number of tubes which should be made up for the series according to formula  $r = \sqrt[n-1]{1/a}$  in which *n* represents the number of tubes to be made up, 1 represents the maximum concentration to be used and *a* represents the minimum concentration to be used. Multiplying the concentration of each standard by the numerical value of *r* would then give the strength of the next standard to be made up. Similarly by determining the minimum difference which could be distinguished, *r* is calculated and from it *n*, the number of standards justified within a given range. The method has been checked with solutions of safranin, luteol, methyl orange, malachite green and Bismark brown. The series is apparently the same for each of these.

<sup>45</sup> D. W. Horn and Sue A. Blake, *Am. Chem. J.* 36, 195-208 (1906).

<sup>46</sup> David W. Horn and Sue A. Blake, *Am. Chem. J.* 36, 516-21 (1906).

<sup>47</sup> Arthur P. Harrison, *J. Opt. Soc. Am.* 7, 375-6 (1923); *Science* 57, 716-7 (1923).

**Variable Size of Colloidal Particles**—In a much greater number of cases than usually indicated, the problem is in reality one of measurement of a suspended precipitate which is too fine for nephelometric measurement. Sulfide methods for copper and lead are good examples. According to all of our reasoning these salts insofar as they dissolve, are ionized and uncolored or slightly colored. The suspended precipitate is therefore the source of color. Of some 20 inorganic methods picked at random, about 50 per cent will give a precipitate on addition of sufficient reagent to sufficient test substance.

In many cases the procedures used provide for a protective colloid or stabilizer. This may be of the neutral colloid type such as gelatine, gum acacia (arabic), starch or egg albumin, or of the "solution link" type<sup>48</sup> such as hydrogen sulfide, ferric chloride and others. Such protective colloids cause and preserve greater uniformity in particle size where colloidal solutions are being compared colorimetrically. In many cases colorimetric comparison without them would be impossible.

**Dichromatism**—If the color of a solution is composed of two or more maxima of luminosity, and if the rate of change of these maxima is not the same, dichromatism occurs. A typical case is bromocresol purple at pH 7.6.<sup>49</sup> The curve A in Figure 96 is obtained at a low concentration. If the depth of the solution be increased to 10 times that of the original, or if the concentration be multiplied by 10, the curve B is obtained. The curve A represents a blue or blue-green with a red tinge. Curve B is a red.

Another effect is to be predicted for any test substance where one ion and the molecule are colored. If the molecule were yellow and the ion blue, the color of the solution would vary from yellow through green to blue according to the concentration of the solution. It follows that comparison of sample and standard at concentrations differing to any considerable extent would be impossible. A deep column of dilute solution would be blue, a shallower column of more concentrated solution would be green.

**Turbidity**—In a turbid solution there is some light which has entered, not been obstructed by any particle, and passed upward through the entire depth of the column. In addition, some light has entered from the side

<sup>48</sup> Arthur W. Thomas and A. Frieden, *J. Am. Chem. Soc.* 45, 2522 (1923).

<sup>49</sup> W. Mansfield Clark, "The Determination of Hydrogen Ions," 3rd ed., p. 161. Williams and Wilkins, Baltimore, Md. (1928).

and been reflected by the particles, unless the sides of the tube are opaque. Light entering from the side has passed through a lesser depth of column and therefore less absorption has occurred. The color registered by the eye is consequently lighter than it would be if all the light reaching it had traveled through the full depth of the column of solution.

Correction for turbidity by addition of a similar turbidity to the standard, is at best a poor substitute. The standard can be corrected by superimposing a turbid sample according to the Walpole technique, although this, too, has several defects. The accuracy attainable in colorimetric technique with a turbid solution is therefore strictly limited.

**Purity of Reagents**—It is almost unnecessary to add that all reagents used should be free from the test substance. This is usually checked by a blank determination, as no reagent can be considered to be above suspicion until its purity has been proved, when working with such small quantities. In work on iron for example, the following amounts have been reported as found in C. P. reagents.<sup>50</sup>

Hydrofluoric acid—the best quality contained 0.007 mg. Fe per cc.

Silica—0.001 mg. Fe per 1 g. SiO<sub>2</sub>.

Hydrochloric acid—Up to 0.04 mg. of Fe per 100 cc.

**Errors in Artificial Standards**—There is a tendency to use standardized sets of glass standards because of their convenience. If accurately calibrated, they may be entirely satisfactory. Several difficulties are to be noted. It is difficult to secure a permanent standard which will match the test solution over the entire range of dilution. Calibration of glass standards is difficult and expensive. Duplication of standards is difficult. At best, a set of glass standards apply only at a definite concentration of reagent, with interfering substances absent, so that they can be used only if the method is operating perfectly smoothly.

**Importance of Sources of Error**—The sources of error have been discussed at length, mainly to provide a warning as to the necessary factors to be considered in the development of new methods, and to show why the procedure is so exactly defined in many cases. The operator, in using a colorimetric method, can only justify changes from the prescribed procedure when it is known, preferably by experimental determination, that an error will not be introduced by such changes. Many of the methods in the literature do not fully account for all of the possible sources of error.

<sup>1</sup> H. L. Smith and J. H. Cooke, *Analyst* 51, 503-10 (1926).

## CHAPTER VIII

### NEPHELOMETRY, PHOTOMETRY AND TURBIDIMETRY

A COLORED material in true solution is estimated by comparison of color with that of a standard. This is a true colorimetric method. The transmission of light is measured and reflection is zero. On the other hand, a colloidal dispersion of a colored precipitate colors the transmitted light and also reflects some light. On the assumption that its reflection is

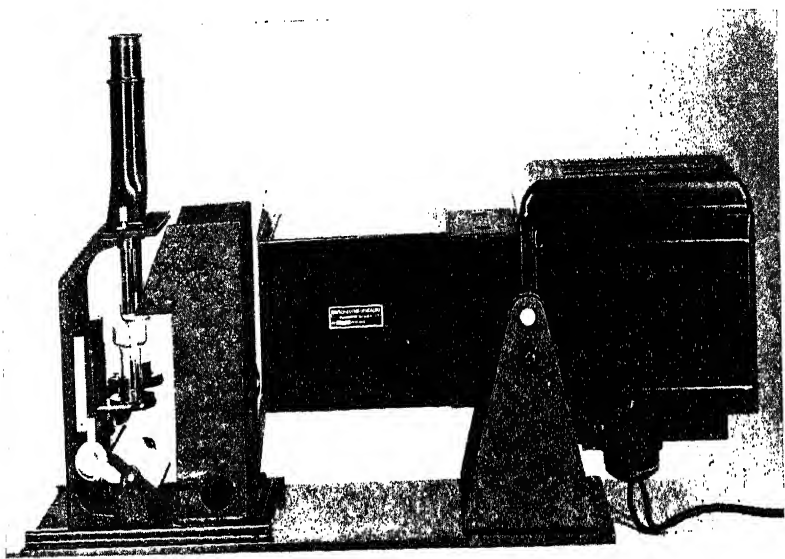


FIG. 57

Duboseq Colorimeter With Nephelometer Attachment. (Courtesy of Bausch & Lomb Optical Co.)

negligible, it may be estimated by colorimetric methods. If the reflection becomes very great the error becomes serious. In that case the reflection of light may be measured instead of the transmission.

These border line cases justify inclusion of nephelometry in a collection of methods which are mainly colorimetric. The same instrument suitably modified may be used for both. Some nephelometric methods



are not in any way borderline cases as the reflection is from a white precipitate which could not be measured colorimetrically.

**Development of Nephelometer**—The earlier development of the nephelometer was largely by T. W. Richards<sup>1,2,3</sup> in connection with atomic weight determinations by comparison with silver. The historical development has been summarized by Kober and Graves.<sup>4</sup>

**Nephelometric Equipment**—The simplest nephelometric equipment is, like that of colorimetry, a series of standard tubes. In general, nephelometric standards change with time due to growth of size of particles, so that the standards and sample must usually be prepared at the same time. A protective colloid modifies this requirement to some extent.

The standard type of nephelometer is a modification of the Duboscq instrument, the Kober nephelometer. In the usual nephelometer the opaque tubes with clear glass bottoms used in colorimetry have been replaced with clear glass tubes with opaque bottoms. An artificial source of light is essential for accurate work. This light shining through the side of standard and sample tubes is reflected upward through the tube and eyepiece. The amount of reflection is controlled as in colorimetry by variation of the depth of liquid underneath the plunger. Unlike colorimetry the relationship is not strictly linear.

Other types of nephelometers are the Bloor<sup>5</sup> and Marshall-Banks.<sup>6</sup> The Richards type has been criticized,<sup>7</sup> as has also the modified Duboscq.<sup>4</sup> In the Kleinmann nephelometer the amount of artificial light delivered to the tubes is varied by a variable aperture for light. Accuracy to 0.5 per cent and a linear relationship is claimed.

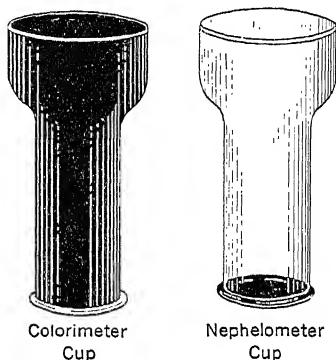


FIG. 58

<sup>1</sup> Theodore W. Richards, *Z. anorg. Chem.* 8, 253-73 (1895).

<sup>2</sup> Theodore W. Richards and Roger C. Wells, *Amer. Chem. J.* 31, 235-43 (1904).

<sup>3</sup> Theodore W. Richards, *Ibid* 35, 510-13 (1906).

<sup>4</sup> Philip Adolph Kober and Sara Stowell Graves, *J. Ind. Eng. Chem.* 7, 843 (1915).

<sup>5</sup> W. R. Bloor, *J. Biol. Chem.* 22, 145 (1915).

<sup>6</sup> J. T. W. Marshall and H. W. Banks, *Proc. Am. Phil. Soc.* 54, 180 (1915).

<sup>7</sup> P. A. Kober, *J. Biol. Chem.* 13, 485 (1912-3).

**Correction Curve for the Nephelometer**—It is necessary to standardize the instrument with solutions of different concentrations and draw a correction curve, or apply a suitable formula. The use of a curve is simpler, but not quite as accurate.<sup>7,10,11</sup>

Prepare various dilutions of standard such as 0.9, 0.8, 0.7, 0.6 and 0.5 times standard. Match against the full strength standard. From these

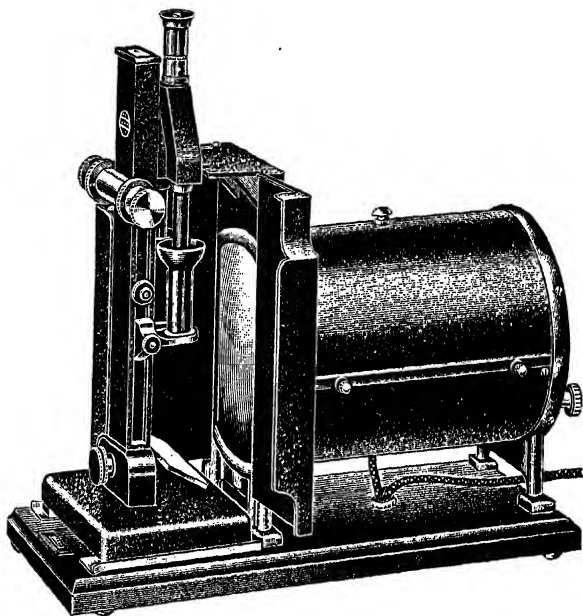


FIG. 59

Kober Nephelometer. (Courtesy of Bimer & Amend)

comparisons the nephelometric constant can be calculated, the several determinations at different concentrations serving as checks. The formula is

$$y = \frac{s}{x} - \frac{(1-x)sk}{x^2}$$

in which  $x$  = ratio of dilute standard to concentrated standard,  $y$  =

<sup>10</sup> P. A. Kober and G. Egerer, *J. Am. Chem. Soc.* 37, 2377 (1915).

<sup>11</sup> Philip B. Hawk, "Practical Physiological Chemistry," 5th ed., p. 294. P. Blakiston's Sons & Co., Philadelphia, Pa. (1916).

height of dilute standard,  $s$  = height of concentrated standard and  $k$  is the unknown nephelometric constant. A more convenient form is

$$k = s(x-1)$$

It is even more convenient to plot the determined values on cross-section paper with readings obtained as ordinates and the ratio of solutions as abscissae. This curve applies for a definite depth of a definite standard. By changing the depth of standard to correspond, when the concentration is varied, the same curve may be used.<sup>12</sup>

**Range of Usefulness**—In sufficiently dilute solution almost any precipitate may be so modified by addition of a protective colloid as to be determinable with the nephelometer. For colored precipitates, finely divided, the colorimetric method is usually preferable.

**Applications**—The applications of nephelometry are numerous. It has been used for measurement of the growth of bacteria in nutrient solutions, and for the protein-thiosalicylic acid precipitate. Other uses were comparisons of water cultures of fungi,<sup>13</sup> estimation of the potential vigor of sweet corn,<sup>14</sup> measurement of turbidity in a sterile system,<sup>15</sup> and detection of changes in the colloidal state.<sup>16</sup> More common applications discussed elsewhere are the determination of ammonia, phosphorus, calcium, acetone, fats and silver.

**Photometry**—Like nephelometry this subject can only be briefly discussed in relation to colorimetry. The turbidity of opalescent glass<sup>17</sup> and of liquids<sup>18,19</sup> may be estimated. The density of fog or smoke can be recorded,<sup>20</sup> and evaluation of bone char by this method has been studied.<sup>21</sup> Under the name of the Tyndallmeter an apparatus is proposed for reading

<sup>12</sup> Philip A. Kober, *J. Biol. Chem.* 29, 155-68 (1917).

<sup>13</sup> George Hockenyos, *Science* 68, 459 (1928).

<sup>14</sup> Charles F. Hottes and Walter A. Huelsen, *Science* 65, 576-7 (1927).

<sup>15</sup> Hans Kleinmann, *Biochem. Z.* 234, 24-38 (1931).

<sup>16</sup> Hans Kleinmann, *J. Lab. Clin. Med.* 12, 629-43 (1927).

<sup>17</sup> W. Ewald, *Z. angew. Chem.* 40, 32 (1927).

<sup>18</sup> Wm. G. Exton, *Proc. Soc. Exptl. Biol. Med.* 21, 181-2 (1924).

<sup>19</sup> Franz Schmidt and Haensch, German Patent 527,895 (1929).

<sup>20</sup> J. S. Fry and Sons, British Patent 237,948 (1925).

<sup>21</sup> C. E. Coates, *J. Ind. Eng. Chem.* 14, 295-8 (1922).

## 94 NEPHELOMETRY—PHOTOMETRY AND TURBIDIMETRY

soil dispersoids<sup>22</sup> and colloids.<sup>23</sup> At high concentrations the reading is no longer proportional to concentration.<sup>24</sup>

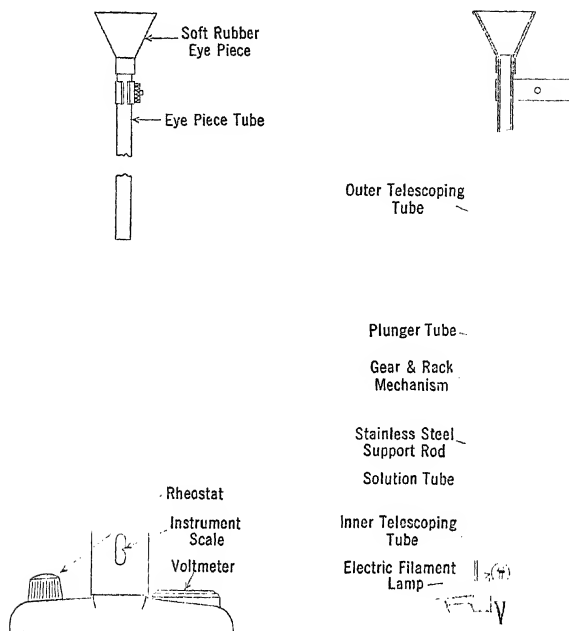


FIG. 60

Burgess-Parr Turbidimeter

Various methods for reading the color of paints or paint pigments with the photometer have been described.<sup>25-29</sup>

<sup>22</sup> F. M. Seales and Franklin W. Marsh, *J. Ind. Eng. Chem.* 14, 52-4 (1922).

<sup>23</sup> S. E. Sheppard and Felix A. Elliot, *J. Am. Chem. Soc.* 43, 531-9 (1921).

<sup>24</sup> R. C. Tolman et al., *J. Am. Chem. Soc.* 41, 297-303 (1919).

<sup>25</sup> J. E. Booge and H. E. Eastlack, *Paint, Oil and Chem. Rev.* 77, No. 15 et seq. (1924).

<sup>26</sup> H. A. Gardner and P. C. Holdt, *Paint Manufs. Assoc. of U. S., Circ.* 173, 153-82 (1923).

<sup>27</sup> J. G. Thompson, George Sutherland and R. F. Gnaedinger, *Ibid.* 184, 272-306 (1923).

<sup>28</sup> H. A. Gardner and H. C. Parks, *Ibid.* 191, 58-140 (1923).

<sup>29</sup> A. E. Bawtree, *J. Oil Colour Chem. Assoc.* 11, 61-70 (1919).

**Turbidimetry**—This may be taken as a special case of nephelometry. Instead of measuring the reflection of light, the depth necessary to obscure a light source to a definite degree is measured. The method is in standard use with the Jackson turbidimeter for determination of sulfur in bomb washings or in water samples. It is also applicable to calcium, or to turbidity in water.

One form of this instrument,<sup>30</sup> shown in Figure 60, is totally enclosed and has an electric bulb as the source of light. To operate, pour the solution into the turbidimeter and insert the plunger tube. Put the eyepiece tube and eye shield in place. Adjust the rheostat on the base of the turbidimeter until 3 volts is shown on the voltmeter. Adjust the depth of the tube by the rack and gear mechanism until the filament either just disappears, or just appears. The former is more convenient. The scale will then give the reading of the depth directly.

As a modification<sup>31</sup> the stationary tube is replaced with a 13 mm. hard rubber tube having an optical glass bottom. Similarly the inner plunger tube is replaced by an 8 mm. one of rubber. By this change only 25 cc. of sample containing 0.7 to 1.3 mg. of sulfur are required.

For low turbidities a special instrument is used.<sup>32</sup> This depends on the use of a blue light source underneath the turbidity tube, and a white light for reflection from the suspended particles. It has been refined to the point where 0.1 p.p.m. of turbidity is readily detected by comparison with standards. Another form of turbidimeter<sup>32a</sup> is based on the width of a calibrated slit necessary to match the Tyndall effect of the solution. Since it is applied only to sulfates it is described with the special method<sup>32b</sup> which uses it. The instruments used are simple modifications of colorimeters.

The photoelectric cell has been applied to measurement of turbidity,<sup>33</sup> and will even ring an alarm when the turbidity goes too high.<sup>34</sup> It has been proposed to record turbidity in absolute units,<sup>35</sup> and to measure that of sugar products by direct comparison of the transmittancy against

<sup>30</sup> S. W. Parr and W. D. Staley, *Ind. Eng. Chem., Anal. Ed.* 3, 66-7 (1931).

<sup>31</sup> K. S. Kemmerer and P. W. Boutwell, *Ind. Eng. Chem., Anal. Ed.* 4, 423-5 (1932).

<sup>32</sup> J. R. Baylis, *Ind. Eng. Chem.* 18, 311-2 (1926).

<sup>32a</sup> R. T. Sheen, H. L. Kahler and E. M. Ross, *Ind. Eng. Chem., Anal. Ed.* 7, 262-5 (1935).

<sup>32b</sup> See pp. 615-7.

<sup>33</sup> P. Jakuschoff, *Z. Ver. deut. Ing.* 75, 426-8 (1931).

<sup>34</sup> Leroy H. Scott, *Fifth Annual Report of Ohio Conference on Water Purification*, 1925, p. 56.

<sup>35</sup> H. Sauer, *Z. tech. Physik.* 12, 148-62 (1931).

a solution of filtered sample.<sup>36</sup> Beer's law does not hold exactly. Color has no effect on the reading. The functions of a colorimeter and a turbidimeter can be combined in one instrument.<sup>37</sup>

A proposed standard<sup>38</sup> of turbidity is a millimolar suspension of barium sulfate in glycerol. This is prepared by mixing equal volumes of 0.002 *M* barium chloride and 0.002 *M* hydroxylamine sulfate in glycerol. The turbidity registered can then be varied by the depth of the illuminated solution, and results read from a calibration curve.

The turbidity of wash water samples from a water filter has been found to be over 40 per cent lower when read by a candle turbidimeter than it is by the standard platinum wire method commonly used in water analysis.<sup>39</sup> The fuller's earth standard reads the same by both methods. This indicates the necessity for caution in the modification of methods in adopting a new instrument for measurement, without preliminary careful calibration. This is further illustrated by the use of four types, the platinum wire, Jackson candle, suspension standards and Baylis super-turbidimeter at the Atlanta water works, in addition to the floc detector.<sup>40</sup>

One gram of 200 mesh and finer, fuller's earth per liter with a reading of 1000 has been recommended as a standard for measurement of clarity of pharmaceutical preparations.<sup>41</sup>

**Ostwald's Law**—This law specifies that the absorption maximum of a colloidal solution shifts with increasing dispersion toward the shorter wave lengths.<sup>42-54</sup> The applications are not within the scope of this subject.

<sup>36</sup> R. T. Balch, *Ind. Eng. Chem., Anal. Ed.* 3, 124-6 (1931).

<sup>37</sup> Hirsch W. Sulkowitch, *Am. J. Clin. Path.* 2, 309-18 (1932).

<sup>38</sup> J. Avellar de Loureiro, *Biochem. Z.* 239, 310-3 (1931).

<sup>39</sup> Wm. I. Van Arnum, *11th Annual Rept., Ohio Conference on Water Purification* 1931, 25.

<sup>40</sup> Paul L. Weir, *J. Southeastern Sect. Am. Water Works Assoc.* 2, 17-23 (1932).

<sup>41</sup> S. Claman, C. J. Carr and J. C. Krantz, Jr., *J. Am. Pharm. Assoc.* 21, 670-1 (1932).

<sup>42</sup> K. Voigt, *Kolloid-Z.* 15, 84-5 (1914).

<sup>43</sup> O. Meissner, *Physik. Z.* 20, 83-5 (1919).

<sup>44</sup> W. Ostwald, *Z. tech. Physik.* 1, 173-5, 261-71 (1920).

<sup>45</sup> Heinrich Trillich, *Farben-Ztg.* 27, 1721-3 et seq. (1922).

<sup>46</sup> Anon. *Color Trade J.* 11, 114-5 (1922).

<sup>47</sup> Ismar Ginsberg, *Textile World* 63, 2615-9, 2645 (1923); *Color Trade J.* 11, 93-6 (1922); *Textile World* 64, 1519-21, 1539 (1923).

<sup>50</sup> H. Heller, *Z. angew. Chem.* 36, 532 (1923).

<sup>51</sup> Ismar Ginsberg, *Textile World* 65, 2127-32 (1924).

<sup>52</sup> Heinrich Trillich, *Farben-Ztg.* 29, 509-10 (1924).

<sup>53</sup> L. Gerstacker, *Farben-Ztg.* 29, 1550 (1924).

<sup>54</sup> C. Pulfrich, *Z. Instrumentenkunde* 45, 35-44 et seq. (1925).

## CHAPTER IX

### CALCULATIONS

**Series of Standards**—When a series of standards is used the weight of test substance in the unknown may be estimated by the amount in the standard to which it corresponds. The percentage or the weight of test substance per unit volume is then readily calculated from this by dividing by the weight of sample used.

**Dilution Method**—The calculation of results when the dilution method is used is somewhat more complex. The darker of the two solutions is diluted until the colors of the two are identical when observed horizontally through tubes of uniform diameter. At this stage the content of test substance per cc. is the same and the total content of test substance of one is to that of the other directly as their volumes. To illustrate:

Weight of standard used, 0.2 gram

Weight of unknown used, 0.2 gram

Standard contains 0.32 per cent of test substance.

Readings, standard 38 cc., unknown 45 cc.

The weights of the sample and standard being the same in this problem we may eliminate calculation of the weight of test substance in the sample, and proceed directly to estimate the percentage by proportion. This is estimated as follows:

Per cent in standard : Per cent in unknown = Volume of standard :  
Volume of Unknown.

$$\begin{aligned}0.32 \quad x &= 38 : 45 \\ x &= 0.379\end{aligned}$$

Therefore we find that the sample being tested contains 0.379 per cent of test substance.

Calculation of results by the dilution method does not always resolve itself into a mere comparison of percentage values, as frequently a known volume of standard solution with a known content of test substance per

cc. is used as the standard. In that case the weight of test substance in the standard must be calculated. From that the weight in the sample and then the percentage present in the sample can be calculated. The method follows:

Weight of sample used, 2 grams.

Standard used, 20 cc. of a solution containing 0.00002 gram of test substance per cc.

Readings, standard 20 cc., unknown 48 cc.

From the readings above it follows that in this case the color of the sample was darker than that of the standard and therefore the sample was diluted. There would be no change in the method of calculation had the standard been diluted.

Weight of test substance in standard : Weight of test substance in unknown = Volume of standard : Volume of unknown.

Weight of test substance in standard is  $20 \times 0.00002 = 0.0004$  gram.

$$\begin{aligned} 0.0004 : x &= 20 : 48 \\ x &= 0.00096 \end{aligned}$$

Therefore the two grams of sample used contained 0.00096 gram of test substance and the percentage in the sample was 0.048.

**Duplication Method**—Results by the method of duplication are readily estimated. A standard solution is added to a blank containing the same reagents as the sample until the color of the sample is matched. Water and standard are then cautiously added, alternately, until the volume as well as the color of the two solutions is identical. The result as to how much test substance is present in the sample is given by the amount necessary to form an identical standard. The percentage in the sample is then calculated as follows:

Weight of sample, 5 grams.

Standard used contains 0.0002 gram of test substance per cc.

Volume of standard used for duplication, 2.3 cc.

Then the total test substance used in preparation of the duplicate was  $2.3 \times 0.0002 = 0.00046$  gram.

Therefore the sample contained  $0.00046 \div 5$  gram of test substance per gram of sample or 0.000092, which is 0.0092 per cent.

**Balancing Method**—Results obtained by the balancing method are somewhat more complicated to calculate. When the two tubes are balanced



the color observed vertically is the same but the depths of solutions are not necessarily identical. This method is applicable only if Beer's law holds. The tubes are calibrated in mm. of depth, except in a few instruments which read in cc. These values may be substituted for mm. of depth. An example follows:

Weight of sample, 2 grams.

Volume of sample after solution before addition to tube *A*, 50 cc.

Height of sample in tube *A*, 7.3 mm.

Height of standard in *B* to balance, 8.4 mm.

Content of standard per cc., 0.00001 gram of test substance.

When the colors of *A* and *B* are balanced the concentrations of the solutions are inversely proportional to the height of the columns, thus:

Test substance per cc. in *A* : test substance per cc. in *B* = Depth of liquid in *B* : Depth of liquid in *A*

$X : 0.00001 = 8.4 : 7.3$

$X = 0.0000115$  gram test substance per cc. in sample solution.

Then the total test substance in the sample is

$$50 \times 0.0000115 = 0.000575 \text{ gram.}$$

This is 0.0002875 gram per gram of sample and the sample therefore contained 0.02875 per cent of test substance.

The general formula<sup>1</sup> for balancing method calculations is

$$X = \frac{F}{R} \times \times \frac{V_u}{V_s} \times \frac{D_2}{D_1} \times \frac{1}{V}$$

In this

$X$  = Concentration of unknown in same terms as  $S$ .

$F$  = Scale reading of standard in mm.

$R$  = Scale reading of unknown in mm.

$S$  = Concentration of standard in standard terms.

$V_u$  = Volume of unknown solution matched against standard.

$V_s$  = Volume of standard solution matched.

$D_1$  = Volume of unknown taken for analysis.

$D_2$  = Volume to which  $D_1$  was diluted when color was developed.

$V$  = Volume of  $D_1$  taken for color development.

In routine work this simplifies down to  $X = F/R \times S$ , or this multiplied by a simple factor, since the remaining terms are selected for simplicity of calculation.

<sup>1</sup> A. R. Rose, *Proc. Soc. Exp. Biol. Med.* 23, 219-20 (1925).

It is always advisable to select samples, particularly for student use, so that calculations involved are only multiplication of readings obtained, by some simple factor. Manipulation by the balancing method is usually standardized at some setting of the standard and only the unknowns varied. In that case a table or curve can be conveniently used for reading the concentration of test substance directly.

## CHAPTER X

### CARBON

#### CARBON IN STEEL

THIS is one of the oldest methods of colorimetric analysis. The basic principle of the method is that a sample of steel when dissolved in nitric acid shows a brown color which is proportional in intensity to the amount of carbon present in the sample. The method is particularly adapted to routine work where many determinations are made on samples of approximately the same composition. The nitric acid used must be free from hydrochloric acid and chlorine, as the latter produces a yellow color.

Other errors in the determination of carbon by this method are that only the carbon present in the form of carbide is measured, the composition of the brown coloration is not constant, and the presence of many impurities will cause colors which interfere. The carbon present in the steel as graphite, which occurs in high carbon steels, is not acted on by acids. In that case the flakes of graphite will be visible in the solution and the determination should be made by combustion. It is sometimes assumed that in steels treated by the same process the proportion of graphitic carbon to carbide carbon will be the same, and comparison is made even of steels which do contain graphitic carbon. This assumption is questionable. If the previous physical treatment of the sample is unknown, colorimetric comparison is of doubtful accuracy.

Manganese lowers the apparent carbon content but may be disregarded if the amount is less than 1 per cent. Nickel has a similar but much greater effect. If much nickel is present, a green color is produced which is difficult of comparison. Over 1 per cent of silica also produces a green color. In cases where the presence of these substances is known, a standard should be taken which has the same content of the interfering substance as that in the sample. Comparison may then be made. Copper, cobalt and chromium in large amounts interfere.

The following conditions should be met :

1. Sample and standard should be made by the same process.
2. Sample and standard should have the same physical condition so far as this can be secured by mechanical means.

3. Sample and standard should not differ greatly in percentage of carbon.

4. Solutions of standard and sample should be made at the same time under the same conditions.

5. The standard used must be one whose ingredients other than carbon are also accurately known.

The tints resulting from different forms of steel are different, and cannot be matched against each other. Bessemer steel must be compared with Bessemer steel and open hearth steel with open hearth steel. The results obtained are more accurate for mild steels than for hard steels. Blair<sup>1</sup> states that the addition of an amount of water equal to the volume of nitric acid used destroys the color from ferric nitrate.

**Sample**—Drill the cooled or quenched sample and reject all drillings which show blue or rusty spots.<sup>2</sup> All should be of uniform size and free from dust. It is desirable to anneal the sample under conditions which are least favorable to the formation of graphite.

**Procedure**—*Dilution Method.* Select as a standard a steel which has been subjected to the same heat and mechanical treatment as the sample, and which has as nearly as possible the same composition as the sample.<sup>3</sup> Transfer from 0.5 to 1 gram portions of sample and standards to tubes and add to each from 10 to 20 cc. of 1:1 nitric acid. In contrast with this, Johnson recommends not over 0.1 gram of sample and 4 cc. of nitric acid.

Heat with occasional shaking on a hot plate or in boiling water until the solutions are clear. Do not immerse in a water bath below the upper level of liquid in the tube as a film of iron oxide will form on the surface and give a brown color of basic ferric nitrate on dilution. To hasten solution, six tubes or less may be heated close together on a sand bath at 190°. They are preferably covered with a beaker to lessen the evaporation of acid.

Cool, transfer to comparison tubes, dilute each to a convenient volume with water and compare the colors by the dilution method. A convenient method of manipulation is to dilute the standard so that each cc. repre-

<sup>1</sup> A. A. Blair, "The Chemical Analysis of Iron," 8th ed., p. 168. J. B. Lippincott Co., Philadelphia, Pa. (1918).

<sup>2</sup> Charles M. Johnson, *Chemical Analysis of Special Steels, Steel Making Alloys, Their Ores and Graphites*, 3rd ed. John Wiley and Co., New York, N. Y. (1920).

<sup>3</sup> U. S. Steel Corporation, "Methods for the Commercial Sampling and Analysis of Plain Steel," p. 11 (1914).

sents 0.01 per cent of carbon. If the standard were 0.54 per cent of carbon it would then be diluted to 54 cc. Dilute the sample until a horizontal color match is obtained. The volume will give the carbon content by direct reading. The final volume of each must be at least double the volume of acid used.

*Series of Standards Method.* Weigh into tubes one or more samples of 0.5 to 1.0 gram and a sufficient number of standards of similar weight to cover the desired range of expected carbon contents. To each add 10 to 20 cc. of 1:1 nitric acid and heat with occasional shaking on a hot plate or in a boiling water bath. Do not immerse below the upper level of the liquid in the bath. When all are dissolved, remove, cool and dilute with water to a standard volume which is not less than double the volume of acid used. Estimate the carbon content of the samples from the series of standards.

**Standards**—A series of steel standards<sup>3</sup> should be available ranging from 0.07 to 0.70 per cent carbon, with intervals of 0.03 to 0.05 per cent up to 0.30 per cent, and intervals of 0.05 to 0.10 per cent above that. Determine the carbon content of a number of standards by the combustion method. Compare these colorimetrically. If the standards do not agree when compared with each other colorimetrically adjust the value obtained by combustion, to agree with those of a selected colorimetric standard. Thus the carbon content of a standard may be assigned a slightly different value from what was actually obtained by combustion in order to correspond to the colorimetric value. Artificial standards such as a caramel solution<sup>5</sup> or a solution of cobalt, iron and copper chlorides<sup>6</sup> have been proposed. Their use is open to objections discussed in Chapter Six.

## COMBINED CARBON IN IRON

**Procedure**<sup>7</sup>—Transfer 1 gram portions of sample and a similar standard iron to beakers, add 30 cc. of 1:1 nitric acid to each and heat until decomposed. Filter off graphitic carbon and silica, washing the filters with water until the filtrate comes through colorless. Collect the filtrates in 100 cc. volumetric flasks, dilute to volume, and mix. Transfer suitable

<sup>5</sup> H. LeChatelier and L. Bogitch, *Ann. chim. anal. chim. appl.* 22, 196 (1917).

<sup>6</sup> A. A. Blair, "The Chemical Analysis of Iron," 8th ed., p. 170. J. B. Lippincott Co., Philadelphia, Pa. (1918).

<sup>7</sup> U. S. Steel Corporation, "Methods for Commercial Sampling and Analysis of Pig Iron," p. 29 (1912).

volumes to comparison tubes and compare the color, diluting standard or sample with water as necessary.

#### CARBON IN STEEL BY CONVERSION TO CARBON DIOXIDE

Carbon in steel may be estimated by oxidation to carbon dioxide and determination by methods for that compound.<sup>8</sup>

**Procedure**—Burn 0.1 gram of filings in carbon dioxide-free oxygen by the usual combustion process. Aspirate the gases into a vessel and dilute with carbon dioxide-free air until the carbon dioxide content is thought to be less than 0.1 per cent. Determine by the indicator method.<sup>9</sup>

#### CARBON IN ORGANIC MATTER BY CONVERSION TO CARBON DIOXIDE

Carbon present in organic samples may be estimated by oxidizing it to carbon dioxide and collecting the evolved gases. Sodium chlorate and 50 per cent sulfuric acid are used as the oxidizing agents<sup>10</sup> by a modification of a procedure for determining nitrogen.<sup>11,12</sup> The same sample can be used for estimation of nitrogen. The carbon dioxide is estimated from its effect on the sodium salt of phenolphthalein.<sup>13</sup>

**Sample**—The sample taken should contain at least 10 mg. of carbon and 0.5 mg. of nitrogen in order to be suitable for estimation of carbon and nitrogen respectively. It should not be so large as to require more than 5 grams of sodium chlorate for oxidation.

Transfer the finely divided weighed sample to the bottom of a 500 cc. Kjeldahl flask. The apparatus to be used is shown in Figure 61. Add sufficient sodium chlorate to the sample to oxidize it and put the flask in place in the apparatus. Normally 1–2 grams are required. Stopcocks B and D should be open and all connections tight. Flask A contains about 100 cc. of water. Flask C has a volume of 2 liters and is filled with 1 per cent sulfuric acid. Flask E also is of 2 liters capacity. Add 25 cc. of 1:1 sulfuric acid to the sample and immediately connect the Kjeldahl flask. Promptness in making this connection is particularly important if the sample contains carbonate as well as organic matter.

<sup>8</sup> A. P. D. McClean and R. B. Denison, *S. African J. Sci.* 23, 257 (1926).

<sup>9</sup> See p. 120.

<sup>10</sup> E. M. Emmert, *J. Assoc. Official Agr. Chem.* 16, 424-7 (1933).

<sup>11</sup> *Ibid* 13, 146-8 (1930).

<sup>12</sup> *Ibid* 12, 240-7 (1929).

<sup>13</sup> *Ibid* 14, 386-9 (1931).

Heat the sample rapidly at first with a high flame so that the air above the sample will be heated and any chlorine peroxide formed will decompose at once. When gases begin to be evolved, lower the flame or even remove it altogether for a time. Resume more active heating as soon as rapid evolution of gas ceases. Continue the heating until the gas has been evolved, the water distilled over from the sulfuric acid, and the latter is refluxing on the sides of the flask. This usually requires 10–15

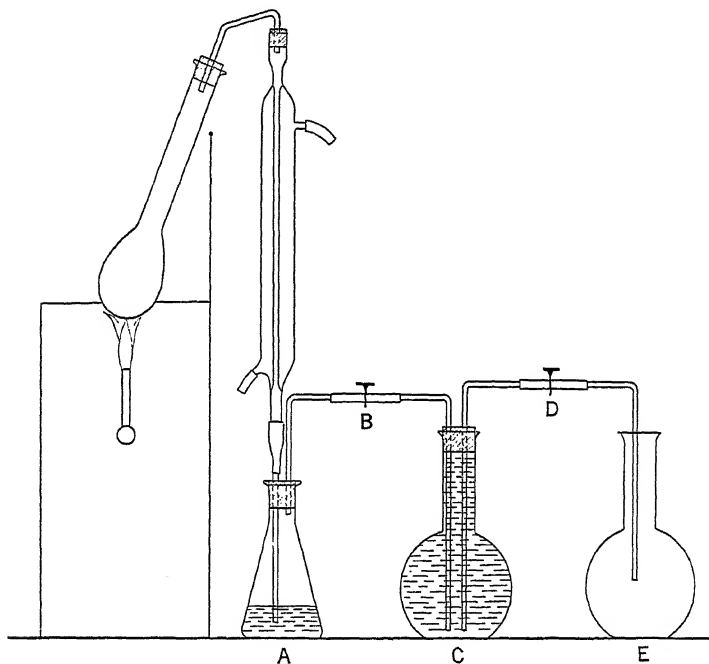


FIG. 61

Apparatus for Decomposition of Sample to Determine Carbon and Nitrogen

minutes. Disconnect the Kjeldahl flask before removing the flame. Wash the condenser into A until it is filled to the stopper. Close stopcocks B and D. The nitrogen is now in A as nitric acid and the carbon in C as carbon dioxide mixed with air. The volume of gas in C is to be measured by the volume of acid displaced into E.

Detach C from A and E retaining the tubing and stopcocks with it. Apply suction at D until a slight vacuum is created in C and close D again. Attach D to a glass tube extending into 10 per. cent potassium

iodide solution in a graduated cylinder. Let in 10 cc. of this solution for each gram of sodium chlorate used and again close D. Shake C intermittently but not violently for 5 minutes. Chlorine is extracted from the mixture of air and carbon dioxide in C, and iodine is liberated. Restore normal pressure in C by opening B. Measure the acid in E to determine the volume of gas in C.

**Procedure**—This requires reference to the method for “Carbon Dioxide by the Sodium Salt of Phenolphthalein” on page 121. Connect C to the apparatus shown in Figure 62. The tube to D is now above the liquid in C. Put a small volume of water in G. Fill flask H and cylinder

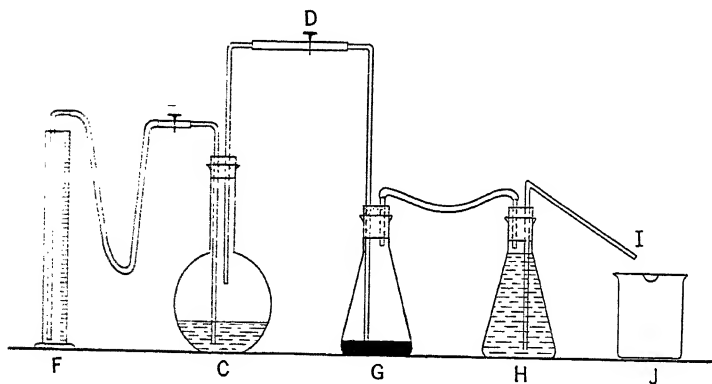


FIG. 62

Apparatus for Estimation of Carbon Dioxide in Mixed Gases

water. Open B and D and start a siphon by applying suction at I.

Remove the water from G and replace with the proper volume of solution of the sodium salt of phenolphthalein. Lower the level of the water in F to that of the water in C. Open D to equalize the pressure in C. Measure the exact volume of water in F with the tube at the water level. Raise F and gas will be forced from C into G. Water will flow from F into C and from H into J. After 70–80 cc. has passed over, again lower F

loss in volume is the gas which

interm vigorously for 5–10 minutes, at

of G should occur. By the use of tubing this can be done without disturbing C or H.

Compare the color of the sodium salt of phenolphthalein by the method



described on page 121 in order to estimate the total amount of carbon dioxide in the volume of gas passed into G. Divide this amount of carbon dioxide in mg. by the volume in cc. of gas used, to give mg. of carbon dioxide per cc. of gas. Repeat the determination of carbon dioxide in the gas until checking results are obtained. Results on the first two aliquots are apt to be low. When an accurate value has been obtained multiply by the volume of gas in C after oxidation and calculate to carbon in the original sample.

## CHAPTER XI

### CARBON MONOXIDE

#### CARBON MONOXIDE BY HOOLAMITE

AN ABSORBENT for carbon monoxide called Hoolamite,<sup>1,2</sup> has been developed. It is prepared by mixing fuming sulfuric acid with iodine pentoxide and an inert supporting material in such proportions that carbon monoxide reacts with the mixture to form a green substance.

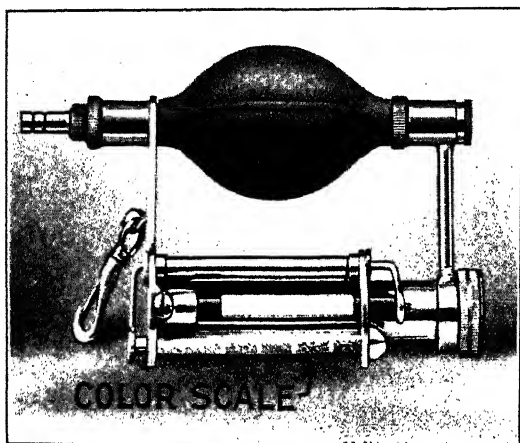


FIG. 63

Carbon Monoxide Detector. (Courtesy of the Mine Safety Appliance Co.)

Other reducing gases such as unsaturated hydrocarbons, hydrogen sulfide, arsine and hydrocyanic acid produce a similar effect but may be removed by passing the sample of mixed gases over a column of active charcoal. Hydrogen, methane, sulfur dioxide, ammonia, nitric acid and other common gases are without action. The green color disappears in a few minutes, so that the same reagent can be used several times.

<sup>1</sup> C. R. Hoover, *J. Ind. Eng. Chem.* 13, 770 (1921).

<sup>2</sup> A. B. Lamb and C. R. Hoover, U. S. Patent 1,321,061 (1919).

**Sample**—*Manholes and Confined Spaces.* Without the ampoule in place, connect a rubber tube of about 3 mm. diameter to the tip of the instrument shown in Figure 63, and let the tubing hang down into the space to be sampled. Squeeze the bulb at least once for every foot of 3 mm. tubing and correspondingly more for larger tubing, in order to draw sample to the instrument.

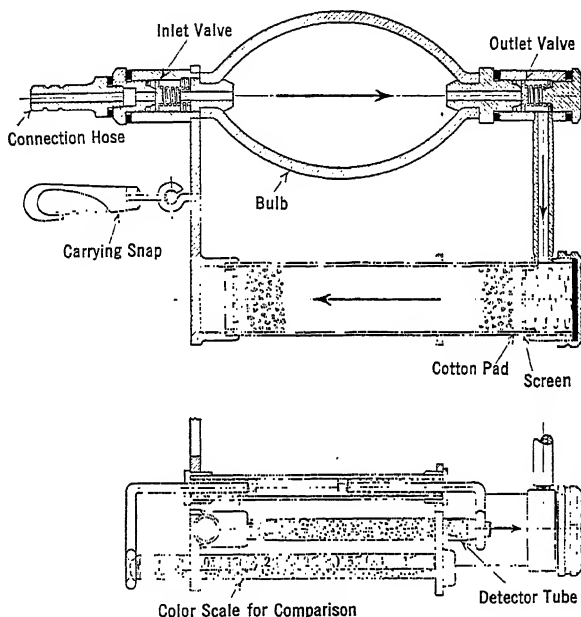


FIG. 64

Diagram of Carbon Monoxide Detector

**Reagent**—Mix 11 parts by weight of iodine pentoxide with 55 parts by weight of fuming sulfuric acid, 80 per cent sulfur trioxide, and add 34 parts by weight of granular pumice stone. When properly protected in closed containers the reagent increases in activity for several days and then remains unchanged, apparently indefinitely. Prepare tubes of reagent 5 mm. in diameter and 50–70 mm. long. The commercial instrument is provided with reagent ampoules. Before use, both tips are broken off at marks previously scored on them and the ampoule inserted in the instrument.

**Procedure**—Pass 500 cc. of the gas sample through the detector in 30–60 seconds. Compare at once with the standards described and estimate the percentage of carbon monoxide present.

*Commercial Detector.* For use of the commercial detector, prior to insertion of the ampoule squeeze the bulb of the instrument several times to make sure that it is filled with the same gas as that to be tested. After insertion of the ampoule squeeze the bulb and allow it to refill 10 times in succession. Compare with the color scale on the instrument. If no color appears after 10 squeezes the carbon monoxide content is less than 0.1 per cent. If no color appears in 20 squeezes the carbon monoxide content is under 0.05 per cent. If color appears in 1 or 2 squeezes the content of carbon monoxide is high enough to be dangerous to the person making the test, unless properly protected by a mask.

**Standards**—Prepare permanent standards in tubes of the same size as used for the test, consisting of granules of pumice stone, normal or basic copper acetate and chromium oxide which match the colors produced by 500 cc. of gas containing 0.03 to 0.2 per cent carbon monoxide according to the procedure outlined. An alternative method is to prepare a scale reading in direct percentage, to be attached to the tube in which the test is made. The commercial instrument has a calibrated scale of standards attached.

**Care of Instrument**—If the color fades from a tube it can be reused. Exhaustion is indicated by a permanent yellow or green color. This is usually after 8 to 10 normal tests. After using, remove the ampoule and place a rubber cap over each end to keep it from contact with air until ready to use again. Leaving it uncapped for 1 to 2 hours will not cause serious deterioration.

The instrument also contains activated carbon to remove gases other than carbon monoxide which would react with the reagent. It must be replaced at suitable intervals, which cannot be specified because of the variability of the nature of exposure.

#### CARBON MONOXIDE BY PALLADIUM CHLORIDE

A simple device <sup>4,5</sup> for testing the air of sewers and confined spaces

<sup>4</sup> L. B. Berger and W. P. Yant, *U. S. Bureau of Mines Report of Investigation*, 3030.

<sup>5</sup> Chester S. Gordon and James T. Lowe, U. S. Patent 1,644,014 (1927).

consists of an easily crushed glass ampoule containing a solution of palladium chloride in water and acetone. On exposure to carbon monoxide the palladium salt is reduced to metallic palladium, giving a yellow or brown to black stain.

When the temperature of the air is above  $10^{\circ}$  the results are semi-quantitative in 10 minutes at 2 to 10 p.p. 10,000. At  $0^{\circ}$  20–30 minutes are required and at  $-16^{\circ}$  a satisfactory result was not obtained in 30 minutes. Ampoules from two different manufacturers varied, one lot giving high readings, another low readings.

Gasoline vapor, ethylene, hydrogen and hydrogen sulfide also give the test but the concentrations which react are also dangerous.

**Detector** <sup>6</sup>—The detector is a thin-walled glass tube about  $5 \times 37$  mm. In this is sealed a water and acetone solution of palladium chloride. The ampoule is covered with a cotton covering and appears much like an ampoule of aromatic spirits of ammonia or of amyl nitrite.

**Procedure**—Crush the ampoule, which will wet the cotton covering. Lower into the space to be tested, either by a cord or by tying to a stick. If properly protected by a mask a workman may carry it into the area to be tested. Expose for not less than 10 minutes.

Carbon monoxide if present will reduce the palladium chloride to finely divided palladium and give a brownish yellow to black stain, indicating the concentration of carbon monoxide. Compare with color standards. Staining will be somewhat uneven and an area representative of the average staining is to be selected for comparison. It is desirable to expose somewhat longer than the specified 10 minutes as a safety precaution.

A special device <sup>7</sup> developed in Germany accomplishes the same end by using a paper wet with the solution in a tube controlled by a rubber bulb. Several successive tests may be made before the papers have to be renewed.

**Standards**—The manufacturers furnish color charts showing the degree of coloration indicating 1 to 10 parts of carbon monoxide per 10,000 parts of air. Precautions with these are as follows:

<sup>6</sup> Sold by Davis Emergency Equipment Co., Inc., 67 Wall St., New York, N. Y., and by Mine Safety Appliance Co., Pittsburgh, Pa.

<sup>7</sup> Romuald Nowicki, German Patent 537,147 (1930).

Less than 1 p.p. 10,000	Safe.
1-6 p.p. 10,000	Caution. Working period not over 45 minutes with helper at hand.
6-8 p.p. 10,000	Extreme caution. Working period not over 15 minutes with helper at hand.
8-10 p.p. 10,000	Dangerous to enter.

**Use as Protective Warning**—The crushed ampoule may be worn on the clothing or equipment to give warning if the carbon monoxide concentration becomes dangerously high.

#### CARBON MONOXIDE BY HEMOGLOBIN AND PYROGALLIC AND TANNIC ACIDS

Normal blood diluted with water and treated with tannic acid forms a grey suspension. Blood in which the hemoglobin is combined with carbon monoxide remains carmine under these conditions. This is used to determine carbon monoxide both in blood and air.<sup>8</sup> The method has been developed in detail at the Bureau of Mines.

**Sample**—*Air*. Aspirate the air to be examined through a soda-lime tube into a 250 cc. sample bottle.

*Ethylene*.<sup>9</sup> The sample is concentrated by distillation to bring it within the range of sensitivity of the method. Take the sample of ethylene containing carbon monoxide in a small pressure cylinder. The fractionating apparatus as shown in Figure 65 is of glass with fused joints, with the exception of pressure tubing connections *t* and *c* to the pressure cylinder and to the sample bottle.

Bulbs J and G are for condensation of the gas by liquid air in a Dewar flask. Mercury safety manometers K and H show the pressure in J and G. Sample bottle A has a capacity of 300 cc., of which the pressure is shown by the safety manometer E. An additional manometer L serves to test the efficiency of the rotary oil pump connected at P for evacuation of the system.

To operate, put the cylinder in place at *t*. Heat this with hot water to a temperature well above the critical temperature of ethylene. Close all stopcocks on the evacuated system to separate the fractionating bulbs

<sup>8</sup> V. Andriska, *Z. Unters. Nahr. Genussm.* 46, 43-6 (1923).

<sup>9</sup> Harold S. Booth and Madeline B. Campbell, *Ind. Eng. Chem., Anal. Ed.* 4, 131-4 (1932).

and the sample bottle. Raise a Dewar bottle of liquid air around J and open stopcock S and valve V. The ethylene in the cylinder is all in gaseous form and is representative of the contents of the cylinder. When solid material equivalent to 25 cc. of liquid ethylene has condensed in J, close the valve and stopcock S. Allow the ethylene in J to liquefy. When the liquid begins to boil, open stopcocks N, D and B to collect a sample of gas in the bottle A. When atmospheric pressure is reached, as shown by manometers K and E, close B and D and either immerse J in liquid air to prevent further rise in pressure or vent it to the air through O and P.

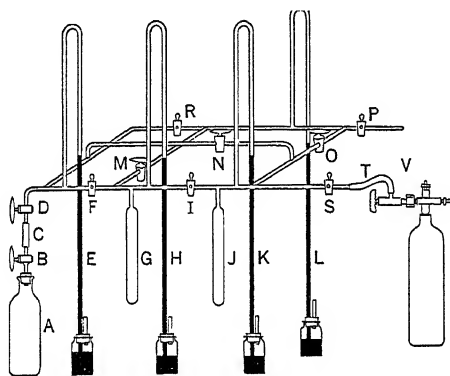


FIG. 65

Apparatus for Fractionation of Carbon Monoxide from Ethylene

All of the carbon monoxide of importance in 25 cc. of a commercial grade of ethylene is contained in the first sample taken. If desired, a second similar bottle may be filled at A as a safety precaution.

**Procedure**<sup>10</sup>—*Blood*. Measure into a test tube of the same size as those used for the standard solutions, 1 cc. of 0.05 per cent potassium citrate or 0.03 per cent sodium fluoride solution, corresponding to the anti-coagulant used in the standard solutions. Draw off 0.1 cc. of blood from the finger of the subject with a capillary pipet and discharge into the test tube. Add 1 cc. of a mixture of equal volumes of 2 per cent pyrogalllic acid and 2 per cent tannic acid solutions, or add 1 cc. of distilled water and 0.02 gram each of tannic and pyrogalllic acids. Mix and

<sup>10</sup> R. R. Sayers, W. P. Yant and G. W. Jones, *Bur. Mines Repts. Investigations* No. 2486 (1923).

let stand for 10 minutes. Compare with prepared standards. At the same time conduct a blank experiment on an unexposed subject.

The method deviates by less than 3 per cent from the spectrophotometric, carmine and Van Slyke methods. It is estimated to give the carbon monoxide in blood with an accuracy of 5 per cent except at very low concentrations.

*Air or Other Gas.* Dilute 0.1 cc. of normal blood to 2 cc. with addition of anti-coagulant and discharge into the sample bottle. Rotate the stoppered bottle for 15 to 20 minutes avoiding shaking. Pour the blood into a test tube, add 0.02 gram each of tannic and pyrogalllic acids and compare with standards after 10 minutes.

The equation for the partial pressure of carbon monoxide requires no connection if the amount of oxygen is below 19 per cent and the temperature is between 18 and 22°.

$$\frac{COHb}{100 - COHb} \times \frac{2093}{300}$$

P<sub>co</sub> = parts per 10,000 parts of air.

COHb = per cent saturation as found by analysis.

2093 = oxygen content of normal air in parts per 10,000.

300 = average relative combining power of hemoglobin with CO compared with its combining power with oxygen.

The accuracy of the determination in gas is estimated at 10-20 per cent.

**Standards—Natural.**<sup>11</sup> Draw 5 cc. of normal blood and add 0.025 gram of potassium citrate if the blood is to be used immediately, or 0.01 gram of sodium fluoride if the blood is to be kept 3 days or more. Divide into equal parts. Dilute a 2.5 cc. portion to 25 cc. with distilled water. Saturate the other portion with 3 to 5 per cent carbon monoxide gas and then dilute to 25 cc. with water. From these prepare a series of mixtures containing 0, 10, 20, 30, etc. to 100 per cent carbon monoxide-hemoglobin. Put 1 cc. of each into narrow test tubes. To each tube add 1 cc. of a mixture of equal parts of freshly prepared 2 per cent pyrogalllic acid solution and 2 per cent tannic acid solution. Mix and pour a little melted paraffin on the surface at once, keeping the tube in cold water. When the paraffin has solidified, place a disc of cardboard on the paraffin and fill with sealing wax. Avoid enclosing any air. The color develops in 10 or 15 minutes and lasts several weeks.

<sup>11</sup> R. R. Sayers and W. P. Yant, *Bur. Mines, Repts. Investigations* No. 2356 (1922).



*Permanent.* A "pyrotannic detector" is sold by the Mines Safety Appliance Co. of Pittsburgh. It has also been recalibrated in terms of carbon monoxide-ethylene mixtures and gave the data in Table 1.<sup>12</sup> As this shows, less than 0.003 per cent of carbon monoxide in ethylene will not develop a color which can be read on this color scale.

TABLE 1.—CALIBRATION OF "PYROTANNIC DETECTOR" IN TERMS OF PER CENT OF CARBON MONOXIDE IN ETHYLENE

Carbon Monoxide in Ethylene	Scale Reading on 'Pyrotannic Detector''
0.5	80
0.4	75
0.3	70
0.2	65
0.1	50
0.05	40
0.02	30
0.005	20
0.003	12
0.001	0
0.0005	0
Blood blank	0

#### CARBON MONOXIDE BY AMMONIACAL SILVER NITRATE SOLUTION

A roughly quantitative method for carbon monoxide in air, in hydrogen, or in technical gas mixtures, may be carried out by noting the time required for the production of a brown color in a solution of ammoniacal silver nitrate when the latter is exposed to the gas under examination.<sup>13</sup> Avoid traces of organic matter in the reagent, as they also produce a brown color. Pure hydrogen and methane do not interfere, nor does ethylene if the concentration is less than 2 per cent.<sup>14</sup> Acetylene reacts with silver to give a bright yellow insoluble compound, but does not have much effect if the proportion of carbon monoxide to acetylene is 10 : 1.

<sup>12</sup> Harold S. Booth and Madeline B. Campbell, *Ind. Eng. Chem., Anal. Ed.* **4**, 131-4 (1932).

<sup>13</sup> H. Kast and H. Selle, *Gas Wasserfach* **69**, 812 (1926).

<sup>14</sup> H. Kast and A. Schmidt, *Gas Wasserfach* **70**, 821-2 (1927).

**Reagent**—Prepare the reagent by dissolving 1.7 grams of silver nitrate in water. Add 36 cc. of a 10 per cent ammonium hydroxide solution, 200 cc. of an 8 per cent sodium hydroxide solution and dilute to 1 liter.

**Procedure**—Put 1 cc. of reagent into a tube 10 cm. long and 1 cm. in diameter. Evacuate and seal the narrow end. Scratch the tube near the end in order that the tip may be easily broken off. Prepare several tubes at a time. When in a room in which the air is to be tested, break off the end of a tube to let air enter, close with the thumb and shake. Estimate the amount of carbon monoxide present by the time elapsed before the appearance of a brown color.

The originators report the following:

Per Cent by Volume of CO	Lapse of Time in Seconds
1.6	7
0.8	10
0.4	20
0.2	35
0.1	45
0.05	80

Other investigators <sup>14</sup> report as follows:

Per Cent by Volume of CO	Lapse of Time in Seconds	
	Air	Hydrogen
1.0	6-8	5-6
0.5	10-12	10
0.3		15
0.25	20-30	
0.12	35-40	
0.10		25
0.06	60-70	60-70

A table should be prepared for each solution, using known quantities of carbon monoxide in the kind of gas to be tested.

This method was used during the war and only occasionally found unsatisfactory.<sup>16</sup> It was found advantageous to make the pointed end of the ampoule very fine or to replace it by a thick walled capillary.

<sup>16</sup> K. Schultze, *Gluckauf* 62, 1496-7 (1926).

## CHAPTER XII

### CARBON DIOXIDE

#### CARBON DIOXIDE BETWEEN 0.03 PER CENT AND 6 PER CENT

THE method consists of dissolving carbon dioxide in a solution of sodium bicarbonate to the point of saturation, and determining the hydrogen-ion concentration.<sup>1,2,3</sup> By the use of the glass electrode a higher degree of accuracy is obtainable, but this requires much more detailed equipment. With due attention to detail, the colorimetric method is usually satisfactory and is rapid and convenient. Comparison may be with pH standards or with colored glasses.

The amount of carbon dioxide dissolved depends on its partial pressure in the sample of air and is independent of the volume of air blown through, provided the solution is saturated with the gas. Varying hydrogen-ion concentrations are shown by the color changes with the indicators. The pH as determined colorimetrically was found to be 0.1 to 0.3 units low, varying with the indicator.<sup>3</sup> This systematic error was constant with a given indicator, so that a calibration curve was prepared for use with glass standards.

Not all indicators are equally satisfactory. The original workers used phenolsulfonephthalein.<sup>1</sup> Another has found bromthymol blue and cresol red or alpha-naphtholphthalein most satisfactory.<sup>3</sup> For less than 4 per cent of carbon dioxide in a gas, continuous reading can be obtained by dropping the alkaline indicator solution from a capillary tube through the gas, collecting the drops and continuously reading the color with a photoelectric cell.<sup>5a</sup> A mathematical analysis of the method has been given.<sup>6</sup> Alveolar air may be collected by the method of Marriott.<sup>7</sup> Other gas samples are taken with the usual bags or pipets.

<sup>1</sup> H. L. Higgins and W. M. Marriott, *J. Am. Chem. Soc.* 39, 68 (1917).

<sup>2</sup> P. W. Wilson, F. S. Orcutt and W. H. Peterson, *Ind. Eng. Chem., Anal. Ed.* 4, 357-61 (1932).

<sup>3</sup> P. W. Wilson, *Science* 78, 462-3 (1933).

<sup>5a</sup> J. Fegler and T. Modzelewski, *Compt. rend. soc. biol.* 116, 248-50 (1934).

<sup>6</sup> E. B. Powers and J. D. Bond, *Ecology* 8, 471-9 (1927).

<sup>7</sup> W. M. Marriott, *J. Am. Med. Assoc.* 66, 1594 (1916).

**Buffers**—Prepare a 1/15 molar acid potassium phosphate solution by dissolving 9.078 grams of pure recrystallized salt,  $\text{KH}_2\text{PO}_4$ , in distilled water. A solution of the salt should show no test for chloride or sulfate. The loss in weight at 20–30 mm. and  $100^\circ$  for 24 hours should be less than 0.1 per cent. On ignition the loss should be  $13.23 \pm 0.1$  per cent. Add 200 cc. of 0.01 per cent phenolsulfonephthalein or other indicator solution and dilute to 1 liter. Standardized indicator containing sodium bicarbonate is not suitable. To make a 1/15 molar alkaline sodium phosphate solution expose to air, protected from dust, the pure recrystallized salt,  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , for 10 days or 2 weeks. The dihydrate,  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ , is obtained. As a check on the purity of this, the solution should be clear and give no test for chloride or sulfate. The salt when dried at 20–30 mm. and  $100^\circ$  for 24 hours and carefully ignited should show a loss in weight of  $25.28 \pm 0.1$  per cent. Dissolve 11.876 grams of this in water, add 200 cc. of 0.01 per cent phenolsulfonephthalein or other indicator solution and dilute to 1 liter.

Mix the two standard phosphate solutions in the proportions indicated below:

Standard	3	7	10	15	20	30	40	50	60
$\text{KH}_2\text{PO}_4$ in cc. ....	5.4	10.5	15.0	20.0	29.0	33.0	40.5	46.5	52.0
$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in cc. ....	94.6	89.5	85.0	80.0	71.0	67.0	59.5	53.5	48.0

Put these solutions into small test tubes, 10 by 75 mm., stopper and seal.<sup>8</sup> Keep them in the dark when not in use. A small amount of thymol or toluene added to each solution prevents the growth of mold.

**Standards**—Prepare two standard solutions of sodium bicarbonate; A, 0.001 *N*, and B, 0.0107 *N*. For A take 10 cc. of 0.1 *N* sodium hydroxide or sodium carbonate solution. Add 200 cc. of indicator solution and dilute to 1 liter. For B take 107 cc. of 0.1 *N* sodium hydroxide or sodium carbonate solution. Add 200 cc. of indicator solution and dilute to 1 liter. Pass carbon dioxide from a cylinder or from the lungs through these solutions in order to convert the alkali to sodium bicarbonate. This conversion need not be quantitative.

**Procedure**—Prepare 20 cc. of the diluted standard solution of sodium bicarbonate. Put 5 cc. of this in a test tube provided with an inlet tube drawn to a fine capillary. Blow in the air sample with an atomizer bulb

<sup>8</sup> The standard solutions and apparatus as used by the U. S. Navy may be obtained complete from Hynson, Westcott and Dunning, Baltimore, Md.

or aspirate a suitable sample through the solution until no further color change occurs. This takes about one minute. Stopper the tube with a paraffined cork and compare the color immediately with the buffer tubes or with color discs for which a calibration curve has been prepared.

By matching the sample against buffer solutions, one may read directly parts of carbon dioxide per 10,000, or hundredths of a per cent, when the standard bicarbonate solution A is used. When the standard bicarbonate solution B is used, the numbers represent parts per 1000 or tenths of a per cent. For the analysis of carbon dioxide higher than 6 per cent, a more concentrated bicarbonate solution is required. The solutions are standardized for temperatures of 20 to 25° and pressures of 730 to 800 mm. Adjustment within that range should be made before reading. The method is inapplicable in the presence of acid or ammonia fumes. Properly conducted, an accuracy to 5 per cent is obtainable, the errors being more in the collection of the sample than in the method of analysis. As outdoor air always contains approximately 0.031 per cent of carbon dioxide, solution A may be easily checked using that as standard gas for testing.

#### CARBON DIOXIDE BY DETERMINATION OF pH

The preceding method depends substantially on comparison with a series of pH standards. Another method is expected to give more accurate results by calculation from the pH of three solutions.<sup>9</sup>

In the equation  $\text{pH} = -n[\log(Kk_{\text{gas}}P) + e]$ ,  $K$  is the primary ionization constant of carbonic acid,  $k_{\text{gas}}$  is the solubility factor of carbon dioxide,  $P$  is the carbon dioxide tension in terms of atmospheres,  $n$  is the rate of change of pH with change in  $-\log(Kk_{\text{gas}}P)$  and  $e$  is a factor dependent on the sample. In the derived form  $\text{pH} = -n(\log P + e)$  and since both  $n$  and  $e$  are unknowns two equations are necessary. These must be at two different carbon dioxide tensions with temperature constant.

**Procedure**—Obtain the pH values <sup>9a</sup> of the following solutions at the same temperature.

1. The original sample.
2. The original sample brought into equilibrium with the carbon dioxide of the atmosphere.

<sup>9</sup> E. B. Powers and J. D. Bond, *Ecology* 9, 364-6 (1928).

<sup>9a</sup> See pp. 697-717.

3. The original sample brought into equilibrium with the breath of the observer.

**Calculations**—From 2 and 3 above calculate the values of  $n$  and  $e$ . Representative partial pressures of carbon dioxide in atmospheres are 0.00031 in atmospheric air and 0.0438 in air expired from the lungs.<sup>9b</sup> Substitute in the equation and solve for  $P$ , using the pH of 1 above. All values are absolute and the usual colorimetric pH standards may be used.

**Sources of Error**—The principal errors are in reading the pH, variations in composition of atmospheric and alveolar air and incomplete equilibria with air. The value in expired air is particularly subject to substantial variation with exercise, physical condition, and other factors. The accuracy can be increased by using a known carbon dioxide mixture in place of the breath and by more accurate determination of pH by electrometric methods.

#### TRACES OF CARBON DIOXIDE

A method similar in principle to the above has been developed for determining carbon dioxide in soil gases or in atmospheric air, of the order of 0.03 per cent. The method depends on the decrease in the color intensity of a solution of sodium hydroxide colored with phenolphthalein, when shaken with gas containing carbon dioxide.<sup>10</sup> A standard solution whose concentration need not be known is placed in the left-hand cup of a colorimeter and the solutions to be compared in the right-hand cup in succession. The left-hand cup is set at some definite reading and kept constant during the comparison.

**Absorbent Solution**—As absorbing solution use 0.005  $N$  sodium hydroxide solution with 0.05 per cent of phenolphthalein in 40 per cent alcohol, added to give the desired depth of color. Make the absorbing solution in a flask fitted with a rubber stopper containing a soda-lime tube and a siphon for delivery.

**Procedure**—As an absorption vessel fit an ordinary gas-sampling tube of about 200 cc. capacity at the top and bottom with 2-way stopcocks,

<sup>9b</sup> Olaf Hammersten and S. G. Hedin, "Physiological Chemistry," 7th ed., p. 863. John Wiley and Sons, Inc., New York, N. Y. (1914).

<sup>10</sup> A. P. D. McClean and R. B. Denison, *S. African J. Sci.* 23, 253 (1926).

each bore being connected to a capillary tube. The vessel should be graduated so that the volume of solution and the volume of gas can be read off. Pass air free from carbon dioxide through the sampler, connect to the flask of sodium hydroxide solution and allow a volume of colored sodium hydroxide solution to enter, equal to the volume of absorbing solution to be used plus the volume of gas sample to be taken. The usual amounts are 50 cc. of sodium hydroxide solution and 50 or 100 cc. of gas, but these volumes depend on the percentage of carbon dioxide in the gas. Connect the upper tube of the sampler to the gas sample and draw off sodium hydroxide solution from the bottom, thus sucking in the desired volume of gas. Shake and compare the color of the solution in a colorimeter and that of a similar solution obtained by shaking the same volume of atmospheric air with the same volume of sodium hydroxide solution, with a comparison solution kept at a fixed level.

**Comparison Solution**—A convenient and stable solution for the left-hand cup is 0.1 *N* sodium carbonate solution colored with phenolphthalein. The amount of indicator to be used depends somewhat on the amount of carbon dioxide absorbed. In analyzing a 25 cc. sample of gas containing about 0.1 per cent of carbon dioxide a suitable standard solution consists of 50 cc. of 0.1 *N* sodium carbonate solution plus 0.2 cc. of 0.05 per cent phenolphthalein in 40 per cent alcohol.

**Standard**—Atmospheric air taken from the open is used as a standard for comparison. Its content of carbon dioxide is remarkably constant, 0.031 per cent.

Instead of calculating the result in the way described above, it is possible to construct an empirical graph obtained by plotting colorimeter differences against percentage of carbon dioxide. For accurate results, for each new sample of sodium hydroxide plus indicator, a fresh graph should be constructed. If only approximate results are desired the same graph may be used if the sodium hydroxide solution plus indicator is made up in exactly the same way.

#### CARBON DIOXIDE BY THE SODIUM SALT OF PHENOLPHTHALEIN

The preceding method has also been adapted to larger quantities of carbon dioxide by estimating the effect on an equivalent mixture of phenolphthalein and sodium hydroxide.<sup>11</sup> The method is essentially a colori-

---

<sup>11</sup> E. M. Emmert, *J. Assoc. Official Agr. Chem.* 14, 386-9 (1931).

metric pH method using phenolphthalein as indicator, but is read directly in terms of carbon dioxide.

An error of 0.1–0.2 mg. of carbon dioxide occurs from the air enclosed in the absorption flasks. This is not significant for amounts of carbon dioxide over 5 mg. Other possible acid vapors are absorbed by 1 per cent sulfuric acid. With a sample containing over 10 mg. of carbon dioxide the error is less than 3 per cent and the majority of estimations were within 1 per cent.

**Sample—Solids and Liquids.** Weigh or measure an amount to give between 10 and 100 mg. of carbon dioxide.

**Concentrated gases.** Introduce a suitable volume by displacement at known temperature and pressure.

**Dilute gases.** Pass a suitable volume of the gas through 30 per cent sodium hydroxide solution as an absorbent and use the solution so obtained as sample.

**Absorbent Solution—**The absorbent must be selected according to the probable amount of carbon dioxide in the sample. The concentrations are given in Table 2. A normal solution of phenolphthalein contains 318 grams per liter.

TABLE 2.—STRENGTH OF SODIUM SALT OF PHENOLPHTHALEIN FOR ESTIMATION OF CARBON DIOXIDE

Amount of Carbon Dioxide To Be Estimated mg.	In 100 cc. Use the Following Strength of Sodium Salt of Phenolphthalein N
2—9	0.0025
9—18	0.0050
18—35	0.0100
35—50	0.0150
50—70	0.0200
70—90	0.0250
90—120	0.0300

In preparation of the absorbing solution, dissolve more than the equivalent amount of phenolphthalein for 2 liters, in 1 liter of alcohol, neutral to phenolphthalein. Dissolve the required amount of sodium hydroxide for 2 liters in distilled water and dilute to 1 liter. Mix the two. For accurate work standardize by titration of a portion with standard acid.

**Procedure—**The apparatus to be used is shown in Figure 66. Place the unknown sample in test tube D, which may be varied in size according



to the sample. Fill the test tube to within 10–15 cc. of the top with water. Put 100 cc. of absorbent solution of the desired concentration in B. Fill C to within 10–20 cc. of the top with 1 per cent sulfuric acid. E and F contain 1:1 sulfuric acid.

At the start stopcock I should be open and all connections air tight. Place the stopper of E in F. Blow in 10–15 cc. of sulfuric acid by opening G and at the same time blowing at N. Close G and replace the stopper and tubes in E without destroying the siphon. If there is a great deal of carbon dioxide in the sample it may be necessary to introduce this acid slowly rather than all at once.

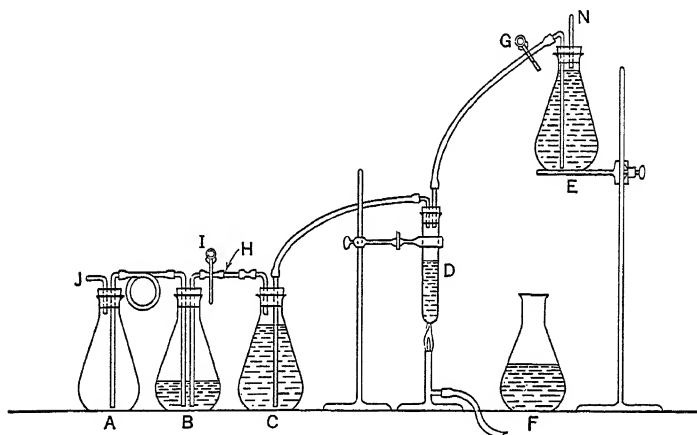


FIG. 66

Apparatus for Collecting Carbon Dioxide Over the Sodium Salt of Phenolphthalein

Heat D until the liquid has boiled for several seconds but has not boiled over into C. Withdraw the flame and immediately open the cock at G. This will sweep gases from D and C by 50 per cent sulfuric acid from E. When this reaches H, which is 100–125 mm. long to permit of ready observation, close I. Make sure that no acid goes through H to B. Disconnect B at H. Lower B below A and shake until B no longer changes color. If the solution becomes colorless or nearly so and there is considerable solution in A apply a suction at J. The vacuum produced when released will drive fresh solution back into B. If the amount of solution in B is small it may be decolorized several times. If both A and B become colorless there is too much carbon dioxide for the amount of absorbing reagent used, or acid from C was carried over into B. To avoid the

latter the tubes at H and all connections must be free from acid before starting.

When no further color change occurs remove the tubes from A and B, mix the solutions and compare with the color of a standard. Prepare this by similar treatment of a suitable volume of a solution containing 24.10 grams of sodium carbonate per liter of distilled water, equivalent to 10 mg. of carbon dioxide per cc.

## CHAPTER XIII

### CYANIDES

#### CYANIDES AS FERRIC THIOCYANATE

CYANIDES present to the amount of 0.03 per cent or less may be estimated by conversion to red ferric thiocyanate.<sup>1</sup> Fluorides, phosphates, arsenates, iodates, oxalates, tartrates and citrates interfere. Acetates and sulfates interfere slightly. Reasonable amounts of chlorides, bromides and iodides are without effect.

**Sample—Inorganic.** If the solution is inorganic, render slightly alkaline with sodium hydroxide solution and dilute to about 50 cc. In dissolving a salt to be analysed for cyanides, the solution must be kept alkaline to prevent loss. This inorganic solution is then used directly.

**Organic.** If the substance is organic, macerate 50 grams of finely ground material with 100 cc. of water. Wash into a distilling flask with another 100 cc. of water. Connect with a condenser dipping into 50 cc. of 4 per cent potassium hydroxide solution. Fit the distilling flask with a separatory funnel containing 50 cc. of concentrated sulfuric acid. When the apparatus is set up and all connections are tight, add the acid to the sample and distil over about 150 cc.

Dilute the distillate to a convenient volume, such as 250 cc., and take a 50 cc. aliquot for the balance of the process.

**Procedure—**To the 50 cc. of solution add 1 cc. of yellow ammonium sulfide and evaporate to dryness on a water bath. This converts the cyanide to thiocyanate. Take up with 10 to 15 cc. of hot water and slightly acidify with 1:3 hydrochloric acid. Filter off the sulfur, add 0.5 cc. of 1:3 hydrochloric acid and boil for 5 minutes. Filter off the sulfur and repeat the boiling and filtering until the final solution is absolutely clear.

An alternative method is to use 5 cc. of a 4 per cent solution of potassium sulfide, instead of 1 cc. of yellow ammonium sulfide. Less trouble will be experienced with sulfur if this is used. Dilute the clear solution to nearly 50 cc., add 15 drops of 5 per cent ferric chloride solution and

---

<sup>1</sup> E. K. Francis and W. B. Connell, *J. Am. Chem. Soc.* 35, 1624 (1913).

dilute to 50 cc. If the iron is precipitated on addition of the ferric solution it indicates that the solution is alkaline and must be acidified. A lemon yellow rather than a red color indicates that the solution is too strongly acid.

Compare in a colorimeter with a standard solution. If the solution is more than faintly acid, results will be low. If too great difficulty is encountered in filtration of the sulfur, repeat that portion of the procedure with another aliquot and after evaporation, extract the residue with 10 cc. of acetone.<sup>2</sup> Rub with a glass rod to facilitate extraction, decant the extract and repeat the extraction twice more. Evaporate the combined acetone extracts to dryness on a water bath and take up with water. Treat this in the same way as the filtered aqueous sulfide extract above.

**Standard**—Prepare a standard solution by dissolving 15 grams of potassium thiocyanate in water and diluting to 1 liter. Standardize gravimetrically with silver until 1 cc. contains 14.92 mg. of potassium thiocyanate. This is equivalent to 10 mg. of potassium cyanide per cc. Dilute this 1 to 10 in using with the ferric chloride reagent, thus making each cc. of the final color equivalent to 1 mg. of potassium cyanide. Compare by the balancing method. Due to fading of the color a series of standards cannot be used. This is probably due to reduction by isodithiocyanic acid, hydrocyanic acid and hydrogen sulfide.

Sample and standard should therefore be prepared at the same time and compared promptly. The duplication method can be used but is more subject to error. Results agreeing well with the silver nitrate method are obtainable with less than 0.5 per cent of hydrocyanic acid.

#### CYANIDES AS PRUSSIAN BLUE

Potassium ferrocyanide reacts with ferric salts to give ferric ferrocyanide, Prussian blue. Although this is insoluble it is obtained so finely suspended as to appear to give a true solution and may be used for colorimetric comparisons.<sup>3</sup> Alkaline salts of the halogens other than potassium fluoride are without effect. The latter is apparently helpful.<sup>4</sup> Reducing agents may interfere.

A quantitative test paper has also been proposed.<sup>5</sup> The paper is wet

<sup>2</sup> M. O. Johnson, *J. Am. Chem. Soc.* 38, 1230 (1916).

<sup>3</sup> E. Berl and M. Delpy, *Ber.* 43, 1430 (1910).

<sup>4</sup> A. Viehoveer and C. O. Johns, *J. Am. Chem. Soc.* 37, 601 (1916).

<sup>5</sup> Georges Magnin, *J. pharm. chim.* 14, 233-6 (1931).

with dilute sodium hydroxide solution and a drop of dilute ferrous sulfate solution. When hydrocyanic acid evolved from acid solution is passed over this test paper the blue color is stated to be proportional to the content of hydrocyanic acid.

**Sample**—The sample may be prepared as in the previous method. Alternatively, if a very dilute solution of cyanide is to be used, adopt the following method. Put a suitable volume of solution in a round-bottom flask. Make it distinctly alkaline with 50 per cent potassium hydroxide solution. Connect with a vacuum pump and concentrate, heating at 50–60° if necessary, by means of a water bath. The final volume will vary according to the salt content but is desirably less than 5 cc.

**Procedure**—To the sample and to a suitable volume of standard cyanide solution add 0.5 cc. of 3 per cent ferrous sulfate solution. For 1 molecule of hydrocyanic acid at least 2 molecules of ferrous sulfate are needed. After standing for 10 minutes with frequent shaking heat to boiling for 2 to 15 minutes, according to the amount of cyanide. When cool, acidify with 10 per cent hydrochloric acid. After some time the original greyish-green precipitate becomes blue and settles to the bottom. After 5 hours, if the supernatant liquid is colorless, dilute each to a suitable volume, such as 100 cc. and shake. The blue suspension is stable long enough to examine in a colorimeter. If the solution above the precipitate is colored, pour it off carefully and suspend the precipitate in distilled water.

Very dilute solutions may not give the reaction. In that case extract the faintly acid solution 8 or 10 times with 1 cc. portions of ether. Then extract the cyanide from the ether with two 1 cc. portions of 1 per cent potassium hydroxide solution. Treat this more concentrated solution as above. In this way as low as 0.04 mg. of hydrocyanic acid per cc. can be determined.

**Standard**—As a standard prepare a 1.0 or 0.1 per cent solution of potassium cyanide and treat in the same way as the sample.

#### HYDROCYANIC ACID AS AMMONIA BY NESSLER'S REAGENT

Either acid or alkaline hydrolysis of hydrocyanic acid gives an ammonium salt. Hydrolysis must be carried out in a closed system to avoid loss since either the starting material or the product is volatile. The am-

## CHAPTER XIV

### OXYGEN

#### OXYGEN BY CUPROUS CHLORIDE

THE properties of the copper chlorides in ammoniacal solution, cuprous chloride being colorless and cupric chloride a deep blue, may be used for the estimation of dissolved oxygen.<sup>1,2</sup> The addition of cuprous chloride to the sample will result in its oxidation to cupric chloride, the blue color being proportional to the amount of available oxygen in the solution. If the sample contained much calcium it would become turbid on the addition of ammonia, therefore add 2 to 3 cc. of a hot saturated solution of ammonium chloride to the sample and standard before addition of the other reagents. If the sample is a sewage effluent or other solution which is slightly colored, add a similar yellow to the standard in the form of an alkaline solution of a yellow dye such as paranitrophenol. The error which will be introduced by the original solution being slightly yellow is not great.

**Sample**—Collect the sample in a bottle and stopper tightly, taking care that there is no air bubble below the cork. A special apparatus<sup>3</sup> is used to transfer this to a tube for examination.

**Apparatus**—The apparatus is made entirely of glass in order to keep the cuprous salt from contact with air. Even coated rubber connections allow some passage of air in the course of an hour or two. In the accompanying figure, A is a buret connected by means of stopcock B, with C, a reservoir for preparing and keeping cuprous chloride solution. E is a buret for supplying ammonium hydroxide. It is connected with C and F by a 3-way stopcock D. F is a mixing bulb for the preparation of pure cuprous ammonium chloride. G is a 3-way stopcock connecting F by means of a capillary with the colorimeter cylinder K. H is a reservoir for holding the sample of water. H contains a tube N which may be connected

---

<sup>1</sup> W. Ramsay and I. Homfray, *J. Soc. Chem. Ind.* 20, 1071 (1901).

<sup>2</sup> W. Ramsay, *Analyst* 34, 193-205 (1901).

<sup>3</sup> G. B. Frankforter, G. W. Walker and A. D. Wilhoit, *J. Am. Chem. Soc.* 31, 37 (1909).

with a Kipp hydrogen generator for replacing the water with hydrogen. K is arranged with a stopcock I, and a stopper through which passes a large tube L, the lower end of which is closed by a disc firmly cemented to the tube. The comparison is made by looking down through this tube.

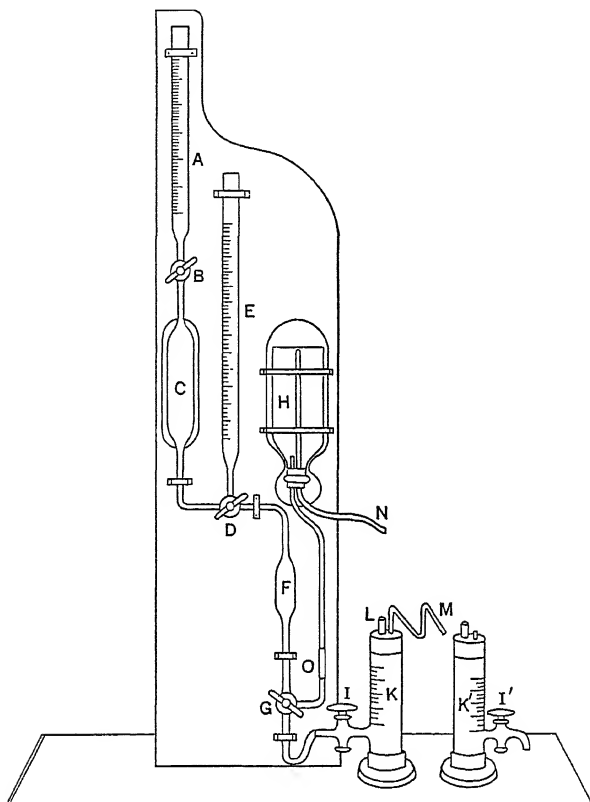


FIG. 68

Apparatus for the Determination of Dissolved Oxygen

M is an outlet for air and water, in completely filling the colorimeter. K' is a similar tube.

**Reagent**—The reagent for the test is prepared by warming a solution of cupric chloride with some granular copper and pouring into water. The white precipitate which forms is filtered and washed, first with hot water, then with alcohol and finally with ether. The powder is dried and must

be pure white. Keeping cuprous chloride free from cupric ion is one of the difficulties of the method.<sup>3</sup> For use, prepare a saturated solution in concentrated hydrochloric acid.

**Filling the Apparatus**—To charge the apparatus, put copper wire into C through A and B. Fill C with the saturated solution of cuprous chloride in concentrated hydrochloric acid and let stand until any cupric chloride has been reduced. Fill A with concentrated hydrochloric acid, to be added as the cuprous chloride is drawn out through D into F, where it is converted into the double salt. Fill F with oxygen-free water from H by opening the 3-way stopcocks G and D. When the water reaches D, close G and D and fill E with ammonium hydroxide. Connect C with F, open B cautiously and cuprous chloride will pass into F as soon as G and I are opened. By noting the height of acid in A, the quantity of saturated cuprous chloride introduced into F may be accurately measured. In charging the apparatus, 2 cc. of the chloride are run in, and 8 cc. of ammonium hydroxide added, the capacity of F being about 10 cc. All the chloride is converted into the double salt. For each determination 2 cc. of reagent are used, made by mixing 0.5 cc. of cuprous chloride and 1.5 cc. of concentrated ammonium hydroxide.

**Procedure**—Completely fill the bottle H with the sample of water, stopper, place in position on the stand and connect with the apparatus at O. Connect tube N with a hydrogen generator. Open I, connect H with K by means of the 3-way stopcock G and completely fill tube K with the sample. Make sure that small bubbles of air which cling to the stopper are removed. K and K' are constructed so that when the stoppers are placed in position, they each hold exactly 102 cc. After the colorimeter is filled with the sample, introduce 2 cc. of cuprous ammonium chloride from F by connecting F with K, opening B, and cautiously opening D. When 0.5 cc. of reagent has been introduced into K, close B and turn D so as to connect E, containing ammonium hydroxide, with K. Introduce 1.5 cc. of reagent into K, making the total of 2 cc. If this is done quickly enough, none of the reagent passes out of the tube M, and there is left in the colorimeter just 100 cc. of sample and 2 cc. of reagent. Close G and I. The colorimeter, which is connected to the apparatus by a flat ground joint and rubber tube, may be removed for the comparison. The blue color appears as soon as the cuprous salt comes into contact with the oxygen in the water.

Place a known volume of standard cupric chloride solution in the



second tube with sufficient ammonium hydroxide to convert the copper into the double salt, and dilute to 100 cc. The volume of standard cupric chloride is chosen so that the color in 100 cc. is deeper than that of the sample. Draw off the standard solution by opening stopcock I', until the color in the two tubes match, when compared vertically.

**Standard**—Dissolve 1.1364 grams of pure copper wire in aqua regia and evaporate off the excess acid. Dissolve in water and dilute to 1 liter. Each cc. is equivalent to 0.1 cc. of oxygen. When 100 cc. of sample water are used in the analysis, the final number of cc. of the cupric chloride standard used for the comparison represents directly the number of cc. of oxygen per liter of sample.

### OXYGEN BY THE STARCH-IODINE COMPLEX

Gaseous oxygen may be determined by its interaction with manganous hydroxide in the presence of potassium iodide.<sup>5</sup> After acidification in the presence of starch, the blue color of the starch-iodine complex is used for comparison. The method is based on that of Winkler, which was first modified for colorimetric application by Rideal and Burgess.<sup>6</sup> They compared the iodine color directly. With the apparatus described below it is possible to detect 0.15 p.p.m. by volume of oxygen.

An apparatus as illustrated in Figure 69, made to withstand a high vacuum, is required for this method. It consists of (1) three reservoirs,  $c_1$ ,  $c_2$  and  $c_3$  of capacity 40, 40 and 20 cc. respectively, (2) mixers A and B, each 45 cc. in volume, and (3) a reaction chamber G, having a capacity of 400 cc. This chamber is connected to  $c_3$  by means of a ground joint, F, and is also furnished with a side tube ending in a ground joint E. E connects the main apparatus to a ground cup of the evacuation leads. The joint is made tight by wax. All stopcocks are well ground vacuum cocks capable of withstanding a vacuum of  $1 \times 10^{-6}$  mm.

**Procedure**—To remove the air entrapped in the bores of stopcocks 1, 2 and 3, evacuate the three reservoirs to 1 mm. by means of an oil pump, with cocks 4, 5 and 7 shut. Close stopcocks 1, 2 and 3, open 4, 5 and 7, turn the two-way stopcock D so as to connect G with E and evacuate as completely as possible, usually to  $1 \times 10^{-5}$  mm. An efficient evacuation outfit consists of a mercury vapor-pump backed by an oil pump. Close stopcocks D, 4, 5 and 7 and detach from the evacuation leads.

<sup>5</sup> P. G. T. Hand, *J. Chem. Soc.* 123, 2573-6 (1923).

<sup>6</sup> S. Rideal and W. T. Burgess, *Analyst* 34, 193 (1909).

To fill  $c_1$  put a solution made by diluting 0.5 cc. of a saturated solution of manganous chloride to 10 cc., into the filler Z. Prepare all solutions with freshly boiled distilled water. Attach pressure tubing, previously soaked in dilute sodium hydroxide solution and then well washed and kept in distilled water, to X and  $X_1$ . Incline the bulb Z so that the solution rests on the bottom of the bulb and connect at Y by means of

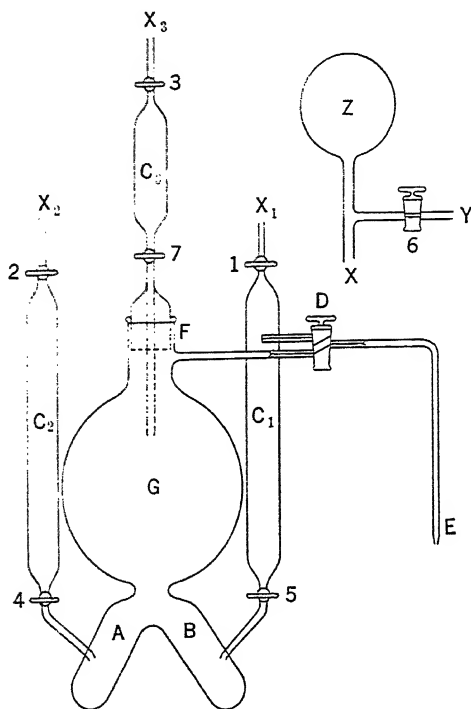


FIG. 69

Apparatus for Estimation of Oxygen by Starch-Iodine Complex

pressure tubing to the oil-pump. Open stopcock 6 and evacuate, letting the solution boil until all air has been expelled from it. Close cock 6 and allow the solution to run into  $c_1$  by first tilting Z and then gradually opening cock 1. Close this cock so as to leave a very small amount of liquid above it. In the same way introduce into  $c_2$  10 cc. of a solution containing 1.5 per cent of potassium iodide and 4.5 per cent of sodium hydroxide.

Connect the apparatus to the supply of gas to be analyzed by fitting

E into a ground glass cup and sealing the joint with wax. Open D to the air and allow the gas to sweep through that part of the apparatus between E and D for half an hour. During this time allow the solutions in  $c_2$  and  $c_1$  to pass into A and B. At the end of half an hour record the temperature of the apparatus and turn the gas flow into G by turning the stopcock D. If care is taken in turning the stopcock the normal rate of flow of the gas need not be disturbed.

As soon as the gas has attained atmospheric pressure in G, as judged by a manometer in the line of the gas flow, close cock D and disconnect the apparatus at E. Mix the solutions in A and B and pour into G by inclining the apparatus. Slowly rock the suspension of manganous hydroxide from side to side at frequent intervals during a period of one hour. At the end of an hour remove the gas in G by attaching the oil pump to E, evacuate the line between D and E and then turn D to connect G with the pump for a short time.

Now fill  $c_3$  in the same way that  $c_1$  and  $c_2$  were filled, with 4 cc. of 20 per cent sulfuric acid and 0.5 cc. of a 1 per cent suspension of soluble starch. After allowing a short time for the mixture to come to room temperature, carefully fill the tubing above stopcock 3 with benzene, attach a small glass Gooch crucible holder to  $X_3$  by means of rubber tubing and fill the holder with benzene. Carefully open stopcock 3 and allow the benzene to flow onto the starch acid mixture, until  $c_3$  is completely filled. With stopcock 3 open, carefully open stopcock 7 so that the solution in  $c_3$  can run into G, care being taken to leave a small amount of the starch-acid mixture above stopcock 7. The oxidized and unoxidized manganous hydroxide goes into solution and the iodine liberated colors the starch. Wash the liquid around the apparatus, transfer to a comparison tube and compare with standards.

**Standard**—Place 1.5 cc. of a 10 per cent potassium iodide solution, 4 cc. of 20 per cent sulfuric acid, and 0.5 cc. of a 1 per cent suspension of soluble starch in a comparison tube and add 1 cc. of a solution of potassium permanganate containing 0.395 gram per liter. Dilute to 24 cc. The blue color is equivalent to that produced by 0.1 mg. of oxygen. Further colors, corresponding to smaller quantities of oxygen, are prepared by using 1 cc. of more dilute permanganate solutions. Suitable standards are 0.1, 0.01, 0.001 and 0.0001 mg. of oxygen. The colors obtained by evacuating the apparatus to a known pressure of air, and hence a known amount of oxygen, are in good agreement with the standard

colors obtained by means of potassium permanganate. A blank test with the apparatus evacuated to  $1 \times 10^{-5}$  mm. should give no color.

#### OXYGEN BY ADUROL

The reagent for this method is a mixture of dry powdered borax and a chlor-derivative of hydroquinone, adurol.<sup>7</sup> The method is only approximate.

**Reagent**—Heat borax for some hours at  $50^{\circ}$ . Mix 6 parts of borax, 1 part of adurol and 3 parts of potassium sodium tartrate, Rochelle salt, as the reagent. The Rochelle salt prevents precipitation of calcium and magnesium.

**Procedure**—To glass-stoppered bottles of uniform size add sufficient sample and standards to fill, then about 0.5 gram of reagent per 100 cc. of sample. Stopper and dissolve the reagent by repeated inversions. Allow to stand 5 minutes. A reddish brown color develops. Estimate the degree of saturation by comparison of the sample with standards. The standard must be very nearly the same in color as the sample. Dilution is not satisfactory.

**Standards**—Standard solutions of oxygen in various degrees of saturation are prepared by shaking water with air, allowing the bubbles to rise and diluting with known volumes of water which has been boiled, and cooled in the absence of air. At  $20^{\circ}$ , oxygen is soluble in distilled water to the amount of 9.17 p.p.m.

#### OXYGEN BY PYROGALLOL

The oxygen-supplying power of the soil has been estimated by the diffusion of gas into a porous porcelain cylinder treated with oil, from whence it is led into a bottle containing an alkaline pyrogallol solution.<sup>8</sup> The time is noted during which the color changes from that of one standard solution to that of another corresponding to a greater concentration of oxygen. The results are expressed as oxygen-supplying power in cubic centimeters per hour.

<sup>7</sup> L. W. Winkler, *Z. angew. Chem.* 24, 341 (1911); *Ibid* 26, 134 (1913).

<sup>8</sup> L. M. Hutchins and B. E. Livingston, *J. Agr. Research* 25, 133 (1923).

## OXYGEN BY INDIGO CARMINE

Dissolved oxygen may be determined by the intensity of the blue color developed by action on the yellow leuco-base of indigo carmine. The method<sup>9</sup> is applicable when only 1 cc. of sample solution is available, and may be carried out within a few minutes.

Results on solutions containing cultures of protozoa have been obtained<sup>9</sup> with an error of 0.5 to 1 mg. of oxygen per liter when 1 cc. of sample was used. By using 10 cc. instead of 1 cc. the error is greatly reduced. Attempts to use the leuco-base of methylene blue by a similar procedure were reported to be unsuccessful.

**Reagent**—Prepare a solution of the reagent by adding sufficient glucose and potassium carbonate to a 0.1 per cent solution of indigo carmine to give a 1 per cent solution of each. Close the flask without having an air bubble under the stopper and put in the dark for 24 hours, or warm on a water bath. The solution develops first a red and then the yellow color of the leuco-base. Discard a small amount of the reagent and pour a thick layer of paraffin oil over the surface. The reagent develops a blue layer next to the oil from the oxygen dissolved in the latter, but this colored layer disappears on standing.

In using the leuco-base, quickly draw 2 or 3 drops of oil into the pipet from over the surface of the reagent, draw in leuco-base, and then draw a drop of oil into the tip to protect the reagent from the air. Make the transfer from stock to sample as quickly as possible. Take somewhat more of the leuco-base than needed so that, if a colored surface develops in emptying the pipet, it will not be introduced into the sample.

**Procedure**—Into a tube from 0.3 to 0.5 cm. in diameter and 5 cm. high, siphon 1 cc. of the sample solution, letting it rise from the bottom of the tube without shaking. Pour on the surface a layer of oxygen-free paraffin oil, taken from over the leuco-base. Introduce quickly beneath the oil by means of a narrow pipet 0.3 cc. of leuco-base. Deliver this as quickly as possible. Mix carefully by introducing and turning a thin glass tube or rod with a small bulb on the end. Compare with standard tubes against a reddish yellow background.

**Standards**—In preparing standard solutions of indigo carmine use tubes of the same size as used for the sample. Dissolve 0.1 gram of indigo

<sup>9</sup> W. W. Efimoff, *Biochem. Z.* 155, 371-5 (1925).

carmine in 100 cc. of water. Pour successive amounts of 3, 2.5, 2, 1.6, 1.2, 0.8, 0.4, 0.3 and 0.2 cc. of this into ordinary tubes and dilute each to 10 cc. with water. Pour the solutions into the narrow comparison tubes described above. These colors may be standardized to correspond to mg. or cc. of oxygen per liter by making determinations on several solutions containing different amounts of oxygen, checking the colorimetric results for greater accuracy with those obtained by the volumetric method of Winkler.<sup>11</sup>

#### OXIDIZING POWER AS OXYGEN, BY CITRIC ACID AND AMMONIUM MOLYBDATE

The active oxygen in oxidizing compounds may be determined by the orange color produced with citric acid and ammonium molybdate.<sup>12</sup> The sample solution must be colorless.

**Procedure**—Shake 10 grams of powdered sample with water at 50–60° in a 250 cc. volumetric flask. After 5 minutes cool and treat carefully, to avoid loss from foaming, with an excess of a 20 per cent solution of citric acid. Dilute to the mark. Add 1 gram of diatomaceous earth, mix and filter. Treat 5 cc. of the filtrate in a comparison tube with 5 cc. of 20 per cent citric acid solution and 5 cc. of a 10 per cent solution of ammonium molybdate. Compare by dilution to match a known solution of sodium perborate treated with the same reagents, or with potassium dichromate solution which has been diluted to match a developed standard solution of sodium perborate.

#### OXYGEN BY OXIDATION OF NITRIC OXIDE

Traces of oxygen such as are given off by respiration of nerves may be estimated by running a measured sample of the gas into an excess of nitric oxide, NO, in the presence of sodium hydroxide solution.<sup>13</sup> The amount of nitrite absorbed is determined by the usual methods for nitrite and calculated to oxygen.

<sup>11</sup> L. W. Winkler, *Z. angew. Chem.* 24, 342 (1911).

<sup>12</sup> R. Jungkunz, *Seifensieder Ztg.* 51, 463 (1924).

<sup>13</sup> H. M. Sheaff, *J. Biol. Chem.* 52, 35 (1922).

## CHAPTER XV

### HYDROGEN PEROXIDE AND OZONE

#### HYDROGEN PEROXIDE BY OXIDATION OF FERROUS IRON

HYDROGEN peroxide may be determined by oxidation of ferrous to ferric iron. The amount oxidised is estimated by addition of a thiocyanate.<sup>1</sup> A concentration of 0.0001 per cent of hydrogen peroxide may be determined. A large excess of thiocyanate is essential to estimation of small amounts of hydrogen peroxide. The method has been criticized as no more than qualitative.<sup>2</sup>

**Reagent**—Dissolve 10 grams of ferrous ammonium sulfate,  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ , in 90 cc. of boiled water free from oxygen and 10 cc. of concentrated sulfuric acid. Test with potassium thiocyanate to be sure that all iron is in the ferrous condition. If not, prepare a neutral solution in 90 cc. of water, reduce with hydrogen sulfide, and remove the hydrogen sulfide with oxygen-free carbon dioxide. Dilute to 100 cc. with oxygen-free water. This reagent must finally be available in a freshly reduced condition, free from reducing agents.

**Procedure**—Add 1 cc. of reagent to 10 cc. of sample in a test tube containing a layer of kerosene. Then add 2 cc. of a colorless 10 per cent solution of potassium thiocyanate and stir with oxygen-free carbon dioxide. This has preferably been washed by bubbling through neutral ferrous sulfate solutions. The color produced may be compared with a series of standards.

**Standards**—Dissolve 0.964 gram of ferric ammonium sulfate,  $\text{Fe}_2(\text{SO}_4)_3(\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$ , in 80 cc. of water, add 10 cc. of concentrated sulfuric acid and 2 cc. of 10 per cent potassium thiocyanate solution, and dilute to 100 cc. Each cc. of this solution is equivalent to 0.00034 gram of hydrogen peroxide. Dilute with a mixture of 10 parts of oxygen-free water, 1 part of ferrous sulfate reagent and 2 parts of a 10 per cent solu-

<sup>1</sup> F. W. Horst, *Chem.-Ztg.* 45, 572 (1921).

<sup>2</sup> Nelson Allen, *Ind. Eng. Chem., Anal. Ed.* 2, 55-6 (1930).

tion of potassium thiocyanate to the desired concentrations. These fade after a time and must be renewed.

A blank of 9 cc. of water may be used and hydrogen peroxide from a known solution added, followed by the same reagents as were added to the sample. The difficulty with this method is obtaining a solution of known strength of hydrogen peroxide. The commercial solution is approximately 3 per cent when fresh.

#### HYDROGEN PEROXIDE BY AMMONIUM MOLYBDATE

A yellow color is produced by the action of hydrogen peroxide on a molybdate in acid solution.<sup>3,4</sup> Ammonium molybdate in sulfuric acid solution also gives a yellow color in the presence of minute quantities of phosphoric or silicic acid. Citric acid does not have this effect and is the acid used. Variations in concentration of the acid and molybdate may be considerable without affecting the result. Variations in room temperature are also unimportant. Nitric acid may be substituted for citric acid with a reduction of over 50 per cent in sensitivity. In order to obtain a definite and dependable color the reagents must be added in the order given.

Much smaller amounts of peroxide may be determined than by the permanganate titration method. An accuracy of 2 per cent is obtainable within the range of 1 to 4 mg. per 50 cc. sample.

**Procedure**—Place in a 50 cc. volumetric flask 30 cc. of water, 10 cc. of 5 per cent citric acid solution, and 1 cc. of the dilute unknown hydrogen peroxide solution. After mixing, slowly add 1 cc. of 10 per cent neutral ammonium molybdate solution. Dilute to the mark and mix well. The color develops at once.

**Standard**—Prepare a permanent standard by dissolving 0.4 gram of potassium chromate in water and diluting to a liter. This standard corresponds to 0.0549 g. of hydrogen peroxide per liter.

#### HYDROGEN PEROXIDE BY DESTRUCTION OF NITRITE

This method for ozone<sup>5</sup> may be modified to determine hydrogen perox-

<sup>3</sup> G. Deniges, *Bull. soc. chim.* [3] 7, 4 (1892).

<sup>4</sup> M. L. Isaacs, *J. Am. Chem. Soc.* 44, 1662 (1922).

<sup>5</sup> Cf. procedure, p. 141.



ale. In that case a third sample is taken which is not passed over chromic acid.

#### HYDROGEN PEROXIDE BY OXIDATION OF TITANIUM SULFATE

This reaction <sup>6</sup> will detect 1 p.p.m. of hydrogen peroxide in the absence of other oxidizing agents. A detailed method is not available.

#### HYDROGEN PEROXIDE BY LIBERATION OF IODINE

This reaction <sup>6</sup> is sensitive to about 0.1 p.p.m. but no detailed method is available.

#### OZONE BY DESTRUCTION OF NITRITE

The destruction of nitrite by ozone has been utilized for its determination.<sup>8</sup>

**Procedure**—Two samples of air are collected, one having been passed over chromic acid and powdered manganese dioxide, the other over chromic acid only. The manganese dioxide destroys ozone. Shake each sample with 25 cc. of 0.00025 *N* sodium nitrite in 0.001 *N* sodium hydroxide solution and 100 cc. of water per 7 liters of gas sample. The destruction of nitrite is measured by estimation by the usual methods.<sup>9</sup> If nitrogen pentoxide is present it is measured as ozone, their reaction in this case being the same. Usher and Rao suggest that both do not occur together.

#### OZONE BY LIBERATION OF IODINE

About 0.001 mg. of ozone can be detected in air by leading the gas through a wash-bottle containing an alkaline or neutral solution of potassium iodide.<sup>6</sup> The reaction is  $O_3 + H_2O + 2I^- = I_2 + 2OH^- + O_2$ , in which 1 molecule of ozone liberates 1 molecule of iodine. When an acid solution is used, more than 1 molecule of iodine is liberated by a molecule of ozone. Other oxidizing agents must be absent.

**Procedure**—Pass the gas sample through 20 cc. of a 0.01 per cent solution of potassium iodide and 0.01 per cent potassium hydroxide for

<sup>6</sup> Nelson Allen, *Ind. Eng. Chem., Anal. Ed.* 2, 55-6 (1930).

<sup>8</sup> F. L. Usher and B. S. Rao, *J. Chem. Soc.* T111, 799 (1917).

<sup>9</sup> See pp. 644-8.

ent time to give a clear, positive test on addition of starch solution. Record the volume of gas passing in the specified time.

Rinse the contents of the wash-bottle out completely, add 1 cc. of 1 per cent boiled starch paste and dilute to 25 cc. Compare with a series of standards similarly developed from an alkaline solution containing 0.001 mg. of iodine and 0.01 mg. of potassium iodide per cc.

**Standard**—Dissolve 0.1 gram of iodine, 1.0 gram of potassium iodide and 1.0 gram of potassium hydroxide in water and dilute to 1 liter. Dilute 10 cc. to 1 liter. Each cc. contains 0.001 mg. of iodine. As standards, use varying volumes of this solution diluted to 20 cc. with a solution containing 0.01 mg. of potassium iodide and 0.01 mg. of potassium hydroxide per cc. Prepare the latter in the same way as the standard iodine solution, omitting the iodine.

#### OZONE BY FLUORESCEIN

A method by which ozone is measured by its oxidation of the leuco-base to form fluorescein has been developed.<sup>11,12</sup> Nitrous vapors, hydrogen peroxide, traces of chlorine and small amounts of carbon dioxide do not interfere. The method is rapid, sensitive and specific. The fluorescence is stable in alkaline solution.

**Reagent**—Dissolve 1 mg. of fluorescein in 1 cc. of 10 per cent sodium hydroxide solution. Add 10 cc. of saturated sodium hydroxide solution. Reduce by shaking with 1 gram of zinc dust until fluorescence is no longer observed. Filter.

**Procedure**—Add 1 drop of reagent to 10 cc. of 0.5 per cent sodium hydroxide solution in a test tube. Pass air through the solution at not over 15 liters per hour until the color matches that of a standard containing 1 part of fluorescein in 100,000,000 parts of water. The ozone in the air is then calculated on the basis that 1 part by weight of fluorescein is produced by 0.96 part of ozone. This ratio indicates that the oxidation of the leuco-base to fluorescein is proceeding at about 25 times the speed of the reaction of fluorescein with ozone to give a colorless compound.

<sup>11</sup> L. Benoist, *Compt. rend.* 168, 612-15 (1919).

<sup>12</sup> M. S. Egorov, *Z. Untersuch. Lebensm.* 56, 355-64 (1928).

## CHAPTER XVI

### COPPER AND CADMIUM

#### COPPER BY AMMONIA

A SOLUTION of a cupric salt, on adding ammonia, gives an intense blue color of the cupric-ammonium complex, which is proportional in intensity to the amount of copper present. This method is particularly applicable to the determination of traces of copper in ores and slags soluble in nitric acid, but is also useful in the analysis of salts containing a trace of copper as impurity. Nickel, cobalt, and manganese interfere. Molybdenum must be absent.

The greatest accuracy is given when the solution contains 0.01274 mg. of copper per cc. The addition of 0.000016 mg. of copper can be detected at that concentration. If the sample solution is colored only faintly blue with ammonium hydroxide, make slightly acid with dilute sulfuric acid and determine with potassium ferrocyanide. In general the method is applicable to samples containing less than 2 per cent of copper. If the color is greenish, readings will be high but the error is usually within the limits of the method. If very difficult to read, repeat the determination. The colors of copper sulfate and copper nitrate are not identical so that selection of the proper standard is essential.

**Procedure—Iron and Steel.**<sup>1</sup> Weigh 5 grams of steel into a beaker and add 100 cc. of 1:12 sulfuric acid. Boil until the steel is dissolved. Copper, silica and carbides remain insoluble. Filter and wash 3 times with hot water. Place the filter paper in the original beaker and add 30 cc. of 1:5 nitric acid. Boil for 5 minutes, filter and wash with hot water. When cool, make alkaline with ammonium hydroxide, add 20 cc. in excess and dilute to 100 cc.

In the presence of molybdenum oxidize the acid solution of the sample with ammonium persulfate and dilute to about 450 cc. Almost neutralize with ammonium hydroxide and saturate the hot solution with hydrogen sulfide. Filter. Wash with hydrogen sulfide solution, ignite and transfer

---

<sup>1</sup> F. T. Sisco, "The Technical Analysis of Steel," p. 272. McGraw-Hill Co., New York, N. Y. (1923).

the oxides to a beaker. Add 10 cc. of 20 per cent sodium hydroxide solution, washing out the crucible with this reagent, and filter. Wash the residue on the paper, dissolve in 10 cc. of hot 1:1 nitric acid and dilute to 60 cc. Make alkaline with 1:1 ammonium hydroxide, add 20 cc. in excess and dilute to 100 cc. Compare.

*Slag.* Weigh 0.5 to 3 grams and add 10 cc. of concentrated hydrochloric acid and 2 cc. of concentrated nitric acid. Heat on a water bath for a few minutes. Dilute to about 50 cc., render ammoniacal and filter. Wash with hot water and cool. Add 20 cc. of 1:1 ammonium hydroxide and dilute to 100 cc. Compare.

*Tailings.* Heat 1 gram of sample with 5 cc. of concentrated nitric acid and 0.5 gram of potassium chloride for one-half hour. Dilute and treat as for slag.

*Vegetables.*<sup>2</sup> Warm a 10 gram sample in a porcelain dish with 25 drops of concentrated sulfuric acid. Platinum must not be used. Heat until the residue is charred. Break up the mass and extract with two 10 cc. portions of 1:5 nitric acid. Completely ash the acid-extracted residue and dissolve in the nitric acid extract. Add 1:1 ammonium hydroxide to slight excess and 5 cc. of saturated ammonium carbonate solution. Warm and filter. Wash the residue with a warm mixture of 10 cc. of water, 1 cc. of 1:1 ammonium hydroxide and 1 cc. of saturated ammonium carbonate solution. Dissolve the precipitate in 5 cc. of 1:5 nitric acid and reprecipitate as above. Combine the filtrates and render them alkaline with 1:1 ammonium hydroxide. Add 20 cc. in excess and dilute to 100 cc. Compare.

If the amount of copper is very small the ferrocyanide method will give more accurate results. The method was successfully applied to peas, beans and spinach.

*Rubberized Fabrics.*<sup>3</sup> Ash 10 grams of fabric in porcelain. Dissolve the ash in 10 cc. of 1:1 hydrochloric acid and dilute to about 30 cc. Filter from any insoluble residue. Pass in hydrogen sulfide until the precipitate settles. If difficulty is encountered in this, add 0.2 cc. of concentrated nitric acid to give a precipitate of sulfur to help settle out the copper sulfide. Filter and wash with 1:10 hydrochloric acid saturated with hydrogen sulfide.

Dissolve the precipitate from the filter with 2 cc. of hot, concentrated nitric acid. Dilute to 10 cc., add 1 cc. of sulfuric acid and evaporate to

<sup>2</sup> A. Hanak, *Z. Unters. Lebensm.* **59**, 511-2 (1930).

<sup>3</sup> A. Ruthing, *Chem.-Ztg.* **54**, 403 (1930).

sulfur trioxide fumes. Dilute to about 10 cc. and render alkaline with 1:1 ammonium hydroxide. Add 20 cc. of 1:1 ammonium hydroxide in excess and dilute to 50 cc. Compare.

If the amount of copper is too small to read well by this method use the ferrocyanide method starting with the method under textiles at "Render the filtrate and washings faintly acid—" <sup>4</sup> For the maximum sensitivity use the sodium diethyldithiocarbamate method. <sup>5</sup>

**Standards**—Prepare a standard solution by dissolving 0.5 gram of pure copper in 10 cc. of 1:1 nitric acid. Dilute the solution to 500 cc. As an alternative, dissolve 1.9645 grams of pure copper sulfate,  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ , in water and dilute to 500 cc. One cc. of either of these solutions is equivalent to 1 mg. of copper. The selection between these standards is to be made according to the acid used for solution of the sample. In general the sample will have been dissolved in *aqua regia* in which case the nitrate standard is used.

To prepare a series of standards use from 1 to 10 cc. of the standard copper solution. To each standard add 20 cc. of ammonium hydroxide and dilute to 100 cc. Comparisons are conveniently made in bottles of uniform size such as selected-quality 4 ounce oil bottles. It will be necessary to renew standards once a month since, although they do not fade in the light, the ammonium hydroxide present forms a flocculent precipitate with the glass.

For duplication mix 70 cc. of water and 20 cc. of concentrated ammonium hydroxide as a blank. Add the standard from a buret with sufficient water so that the final volume is 100 cc.

Prepare a standard for the dilution or balancing method by diluting 10 cc. of the standard solution to 1 liter with 100 cc. of concentrated ammonium hydroxide and 890 cc. of water. This standard contains 0.01 mg. of copper per cc.

#### COPPER IN CONCENTRATED HYDROCHLORIC ACID

If no other metals whose chlorides are also deeply colored are present, the concentration of a copper solution in concentrated hydrochloric acid may be determined by the intensity of the yellow color produced. <sup>6</sup> The intensity of color reaches its maximum at an acid concentration of 28 per cent and after that remains constant. Free chlorine does not interfere

<sup>4</sup> See p. 147.

<sup>5</sup> See p. 164.

<sup>6</sup> C. Hüttner, *Z. anorg. Chem.* 6, 351 (1914).

but nitric acid or nitrogen oxides do. Cobalt or nickel will have very little color in the concentrated acid solution unless the amount is large. Manganese does not interfere. Minute amounts of organic matter may produce a yellow color and if present must be burned out before dissolving in acid. Iron has a yellow coloration which is to that of copper as 9:5. This method is as accurate as the ammonia method and is much easier to apply in some cases.

**Procedure**—Select a sample that contains about 0.01 gram of copper. Dissolve this sample in concentrated hydrochloric acid if possible. If the sample does not dissolve in hydrochloric acid readily dissolve in 1:1 nitric acid or *aqua regia* and add hydrochloric acid to the solution. Evaporate to dryness, add more hydrochloric acid and repeat the process twice. Dissolve the residue in a small volume of 1:1 hydrochloric acid and then add concentrated hydrochloric acid to make a volume of 100 cc. Compare.

If the sample contains interfering metals dissolve in hydrochloric or nitric acid. Add 0.5 gram of potassium chlorate, dilute with water, and precipitate copper as the sulfide by washed hydrogen sulfide. Filter the copper sulfide and ignite. Dissolve the cupric oxide in 10 cc. of 1:1 hydrochloric acid and dilute to 100 cc. with concentrated hydrochloric acid. Compare with a standard.

**Standard**—Prepare a standard by dissolving 0.5 gram of pure copper in nitric acid and expelling nitric acid by hydrochloric acid as directed for the sample. Dissolve in a small volume of 1:1 hydrochloric acid and dilute to 500 cc. with concentrated hydrochloric acid. This standard contains 0.001 gram of copper per cc., or 1 mg. per cc. Because of the concentrated acid used it is not advisable to try to keep permanent standards. The determination may be made by dilution or balancing. If by dilution use concentrated hydrochloric acid as diluent.

#### COPPER BY SALICYLIC ACID

The red color produced by cupric ion with salicylic acid may be used for the quantitative determination of copper.<sup>7</sup> The color produced is not permanent. Free mineral acids, citric and tartaric acids, or more than a trace of iron, interfere. Saccharine, glucose, lactose and invert sugar do not interfere. As little as 0.00001 gram or 0.01 mg. of copper may be detected and estimated by this method.

<sup>7</sup> F. Schott, *Z. Untersuch. Nahr. Genussm.* 22, 727 (1911).

**Sample**—The method is chiefly applicable to examination of food products. The residue from distillation of alcohol from spirits may be ashed, the ash moistened with a mixture of nitric and sulfuric acids, evaporated to dryness and taken up with 1 per cent of acetic acid. Similar procedures on ash from other products should be applicable.

**Procedure**—Prepare a standard copper solution by dissolving 0.392 gram of pure crystallized copper sulfate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , in water and diluting to 1 liter. One cc. of this standard contains 0.1 mg. of copper. Place amounts of this standard varying from 0.1 to 1 cc. in plain test tubes and the sample in aqueous solution in a similar test tube. Dilute each standard to the volume of the sample. Add to each tube 5 drops of a 2 per cent solution of potassium nitrite, 5 drops of 10 per cent acetic acid, and 3 cc. of a 0.5 per cent solution of salicylic acid in 10 per cent alcohol. Heat the tubes to boiling in a water bath for three-quarters of an hour, cool and compare.

Since the color fades, comparison must always be made with standards prepared at the same time as the sample.

#### COPPER BY POTASSIUM FERROCYANIDE

The amount of copper in dilute solution may be estimated from the intensity of the reddish brown color produced by reaction with potassium ferrocyanide. The method is rendered more sensitive by the addition of ammonium nitrate, ammonium chloride or potassium nitrate to the solution. In the presence of these salts the method will show one part in 2,500,000.<sup>8</sup> The method is applicable below 0.05 per cent without taking aliquots, by the use of a sample as small as 1 gram. By means of aliquots, higher percentages of copper may be determined with a lower degree of accuracy.<sup>9</sup>

It is less accurate than precipitation and titration with sodium thiosulfate<sup>10</sup> but is preferred for routine work because of ease of manipulation.<sup>11</sup> The chief source of loss is insufficient washing in transfer of the copper solution prior to addition of potassium ferrocyanide.

<sup>8</sup> W. W. Scott, "Standard Methods of Chemical Analysis," 4th ed., vol. I, p. 198. D. Van Nostrand Co., New York, N. Y. (1927).

<sup>9</sup> R. F. Heath, *Mining Sci. Press* 114, 624 (1917).

<sup>10</sup> R. Hertwig, *J. Assoc. Official Agr. Chem.* 7, 41-3 (1923).

<sup>11</sup> R. M. Mehurin, *J. Assoc. Official Agr. Chem.* 13, 479 (1930); *Ibid.* 15, 541-3 (1932).

**pie—Gelatine.**<sup>12</sup> Weigh 20 to 50 grams of gelatine in a tared platinum or porcelain dish of about 150 cc.<sup>14</sup> capacity. Ash in a furnace previously heated to 500-550°.

When cool, moisten the ash with water, add about 5 cc. of concentrated hydrochloric acid and filter if necessary. If filtered, ignite the residue, dissolve in 5 cc. of 1:1 hydrochloric acid and join with the filtrate. Evaporate to dryness and add 8 cc. of 1:1 hydrochloric acid. Heat to boiling and filter into a 50 cc. flask, diluting to about 40 cc. Heat nearly to boiling, saturate with hydrogen sulfide, stopper and let stand in a warm place for one-half hour. Filter into a 150 cc. flask and wash the precipitate thoroughly with warm 1:20 hydrochloric acid saturated with hydrogen sulfide. Transfer the paper and precipitate to a porcelain crucible and ignite in the furnace at 500°. When cool, moisten the ash with 2 cc. of hot, concentrated nitric acid, add about 2 cc. of water and evaporate to dryness on a steam bath. Dissolve in 10 cc. of 10 per cent ammonium nitrate solution and filter if necessary. Dilute to exactly 50 cc. Measure 10 and 25 cc. aliquots into Nessler tubes. To the 10 cc. aliquot add 3 cc. of 10 per cent ammonium nitrate solution. Dilute both aliquots to 50 cc. but do not mix.

**Lead Carbonate and Pig Lead.**<sup>15</sup> Weigh 30 grams of refined, or 10 grams of crude product, finely divided, into a 400 cc. beaker and add small portions of hot 1:1 nitric acid until the material is dissolved. If basic lead nitrate forms, dilute slightly with warm water and boil. Add 30 cc. of 1:1 sulfuric acid and stir. Decant the supernatant liquid through coarse filter paper, wash by decantation with warm water and finally wash the precipitate onto the paper. Neutralize the filtrate with 1:1 ammonium hydroxide and add 3-4 cc. in excess. Boil a short time and filter. Wash the precipitate with warm water. Render the filtrate just acid with 1:1 hydrochloric acid, add 6 drops of 10 per cent potassium ferrocyanide solution and filter through 2 compact filter papers. Let the precipitate drain without washing. Dissolve the copper ferrocyanide from the paper with alternate washings of hot water and 1:1 ammonium hydroxide, keeping the filtrate to a volume of 30-40 cc. Just acidify, using not more than 2 drops in excess, with 1:1 hydrochloric acid. Transfer to a 50 cc. Nessler tube and dilute to the mark.

<sup>12</sup> R. M. Mehurin, *Ind. Eng. Chem.* 15, 942-3 (1923); *J. Assoc. Official Agr. Chem.* 14, 522-5 (1931).

<sup>14</sup> E. H. Berry, *J. Assoc. Official Agr. Chem.* 9, 458-9 (1926).

<sup>15</sup> B. S. White, *J. Ind. Eng. Chem.* 7, 1035 (1915).



*Red Lead.* Treat 30 grams of finely divided sample with 40 cc. of 1:1 nitric acid and slowly add 30-40 cc. of a 3 per cent solution of hydrogen peroxide, stirring constantly. Dry sodium sulfite may be used in place of hydrogen peroxide. Boil until dissolved, then proceed as with lead carbonate.

*Textiles.*<sup>16</sup> Digest a 10-20 gram sample with 25 cc. of concentrated nitric acid, 25 cc. of concentrated sulfuric acid and 5.0 grams of potassium sulfate in a Kjeldahl flask. When digestion is complete, dilute with water to 75 cc. and neutralize with concentrated ammonium hydroxide. Add 3-4 cc. in excess. Boil a few minutes and filter. Wash the precipitate with warm water and discard.

Render the filtrate and washings faintly acid with 1:1 hydrochloric acid and add 6 drops of 10 per cent potassium ferrocyanide solution. Filter through a close filter paper. Let it drain without washing. Dissolve the precipitate of copper ferrocyanide from the paper by alternately washing with hot water and 1:1 ammonium hydroxide. Be sure the filtrate does not exceed 40 cc. Acidify with 1:1 hydrochloric acid and add 2 drops in excess. Transfer to a 50 cc. Nessler tube and dilute to the mark.

*Vegetables.*<sup>17</sup> For small amounts of copper follow the details of preparation of sample given under the ammonia-complex method,<sup>18</sup> through separation of iron and aluminum, leaving copper in the filtrate. Then go to the previous method for copper in textiles starting with "Render the filtrate and washings faintly acid—"

*Food.*<sup>20</sup> Ash the material after adding 0.2 gram of hydrated lime to prevent loss of copper or lead. Dissolve the ash in 10 cc. of 1:1 hydrochloric acid and evaporate to dryness. Extract the dried residue with 1 gram of citric acid and 2 grams of ammonium acetate dissolved in 5 cc. of water. Dilute to 25 cc. and precipitate by passing in hydrogen sulfide until the precipitate settles readily. Filter and wash on the filter with 1:10 hydrochloric acid saturated with hydrogen sulfide.

Dissolve the precipitate from the paper with 2 cc. of hot concentrated nitric acid. Dilute to 10 cc. and add 1 cc. of 1:1 sulfuric acid. Filter and wash the paper with 5 cc. of 1:20 sulfuric acid. Dilute to 50 cc.

<sup>16</sup> Raphael E. Rupp, *Proc. Am. Assoc. Textile Chem. Colorists* 1930, 215-7; *Am. Dyestuff Repr.* 19, 581-3 (1930).

<sup>17</sup> A. Hanak, *Z. Untersuch. Lebensm.* 59, 511-2 (1930).

<sup>18</sup> See p. 144.

<sup>20</sup> F. W. Richardson, *Analyst* 55, 323-5 (1930).

**Water.**<sup>21</sup> Use the water as sample, concentrating if necessary. Acidify with 1:1 hydrochloric acid until just definitely acid. Exercise all possible precautions to avoid contamination in concentrating.

**Precautions**—If much zinc is present it must be removed before precipitation of the cupric ferrocyanide. In that case make the filtrate from the iron separation slightly acid with acetic acid, add 5 cc. of an 8 per cent sodium ammonium phosphate solution, boil, cool, filter and treat the filtrate as outlined.

If a faint white cloud of lead ferrocyanide appears in the sample add a small amount of a very dilute solution of lead nitrate to the standard.

If the iron precipitate obtained with ammonia is very large, redissolve in 10 cc. of 1:1 hydrochloric acid and reprecipitate with 1:1 ammonium hydroxide. Filter and add the filtrate to the sample. This will recover adsorbed copper removed by the first precipitation of iron and aluminum hydroxide.

**Procedure**—*Series of Standards.* To the 50 cc. sample prepared as above add 0.3 cc. of a 1 per cent solution of potassium ferrocyanide and mix. Compare with tubes containing 2, 3, 4, 5, and 6 cc. of a standard solution of copper sulfate containing 0.1 mg. of copper per cc., to which water and the same reagents have been added as to the sample.

*Duplication.* As an alternative to preparation of a series of standards add to another Nessler tube 5 cc. of 10 per cent ammonium chloride solution, 2 drops of concentrated hydrochloric acid, 0.3 cc. of a 1 per cent solution of potassium ferrocyanide, and 90 cc. of water. Add a standard copper sulfate solution corresponding to 0.1 mg. of copper per cc. until the color matches.

The solution in which the determination is to be made must be nearly neutral. If it is acid the color produced will turn to an earthy brown; if alkaline the color will fade.

**Standard**—Prepare the standard by dissolving 0.3928 gram of pure copper sulfate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , in water and diluting to 1 liter. This standard contains 0.0001 gram or 0.1 mg. of copper per cc.

#### COPPER AS THE SULFIDE

A trace of copper in solution may be determined by formation of the

<sup>21</sup> S. Schoorl, *Z. anal. Chem.* 88, 325-36; *Chem. Weekblad* 29, 338-43 (1932).

brown colloidal sulfide. The amount of copper must not be more than 0.25 mg. and less than half that amount is better. The method is not applicable in the presence of lead, silver, mercury or bismuth. Aluminum, zinc, potassium, sodium and calcium do not interfere.<sup>22</sup> Large amounts of manganese are tolerated.<sup>22a</sup> Addition of ammonium chloride to the solution doubles the delicacy. With proper manipulation accuracy to better than 1 per cent is obtainable.

**Sample—Water.**<sup>23,21</sup> Evaporate 1 liter of water to about 75 cc. and wash into a platinum dish. Add 2 cc. of 1:1 sulfuric acid or enough to neutralize the alkalinity. If much organic matter or clay is present add 5 cc. of the acid. Deposit copper from the solution electrolytically. Dissolve in 1:3 nitric acid and evaporate to dryness on the water bath. If silver is present add a few drops of hydrochloric acid before evaporating. Take up with water, add 5 cc. of saturated ammonium chloride solution and transfer to a Nessler tube.

**Tanning Extracts.**<sup>22</sup> Select a sample to contain at least 0.1 mg. of copper. For tan liquor from a suspender this requires 100 cc. For chestnut or oakwood liquid extracts, about 5 grams are necessary, for other liquid extracts about 20 grams. A sample of solid extract should be about half that specified for a liquid extract. Due to the presence of zinc in some extracts the ferrocyanide method is not applicable.

Take the sample to dryness in platinum and heat gently until only a swollen mass of carbon remains. Add 5 cc. of concentrated sulfuric acid and continue with the addition of small amounts of nitric acid until most of the carbonaceous matter is burned out. Add 1:1 hydrochloric acid and evaporate to dryness on a steam bath. Repeat that operation and then dissolve in 5 cc. of 1:1 hydrochloric acid. Dilute to 50 cc. with distilled water.

Add sufficient concentrated ammonium hydroxide to render the solution alkaline. Boil for a few minutes to coagulate iron and aluminum hydroxides. Filter and wash the precipitate with 1:10 ammonium hydroxide. Reserve the precipitate on the paper for estimation of iron. Boil the filtrate until ammonia is no longer given off and dilute to 50 cc.

**Solutions containing iron.**<sup>24</sup> Acidify a suitable volume of the solution

<sup>22</sup> M. P. Balfe and H. Phillips, *J. Intern. Soc. Leather Trades Chem.* 15, 226-35 (1931).

<sup>22a</sup> L. de Brouckère and S. Solowiejczyk, *Bull. soc. chim. Belg.* 43, 597-625 (1934).

<sup>23</sup> E. B. Phelps, *J. Am. Chem. Soc.* 28, 368 (1906).

<sup>24</sup> A. Castiglioni, *Z. anal. Chem.* 97, 270-3 (1934).

with a definite excess of hydrochloric acid. Add 0.5 gram of sodium hydrosulfite,  $\text{Na}_2\text{S}_2\text{O}_4$ . Boil the solution for 5 minutes. A precipitate of mixed metallic copper and copper sulfide is obtained. Filter and wash the precipitate on the paper. Dissolve the precipitate with the minimum possible amount of hot 1:1 nitric acid. Cool and dilute to 25–30 cc. Add 1:1 ammonium hydroxide until the solution is just ammoniacal and use sample.

*Miscellaneous.* To the sample in solution add 5 cc. of 10 per cent sodium acetate solution, 5 cc. of saturated ammonium chloride solution and 3 cc. of concentrated nitric acid. Transfer to a Nessler tube.

**Procedure—Duplication.** Dilute the sample to 50 cc. and add 10 cc. of a sulfide reagent made by mixing equal volumes of a 10 per cent solution of potassium hydroxide with a saturated solution of hydrogen sulfide. Into a second Nessler tube introduce water, 10 cc. of sulfide reagent and a standard solution of copper sulfate containing 0.1 mg. of copper per cc.,<sup>26</sup> until the colors match in the same volume of solution. If the precipitate shows a tendency to agglomerate and settle, prepare another sample. To this, in addition to the reagents mentioned, add 5 cc. of 50 per cent sugar syrup. This will prevent coagulation of the precipitate.

Conditions for formation of the color of standard and sample must be closely parallel, otherwise they will differ not only in intensity but in hue. This method is often criticised for that reason.

*Series of Standards.*<sup>22</sup> Prepare various dilutions of a standard copper solution containing 0.1 mg. of copper per cc.<sup>26</sup> To 5 cc. of sample and 5 cc. of each standard add 5 cc. of 10 per cent ammonium chloride solution, 3 cc. of concentrated hydrochloric acid and 0.5 of the sulfide reagent. Compare.

#### COPPER BY POTASSIUM ETHYL XANTHATE

Small amounts of copper in solution react with potassium ethyl xanthate to produce a yellow color suitable for colorimetric estimation.<sup>27,27a</sup> The presence of small amounts of iron, lead, nickel, cobalt, zinc or manganese does not interfere. Nickel gives a color very similar to that of copper if present in significant amounts.<sup>28</sup> The use of brass burners may

<sup>26</sup> See p. 150.

<sup>27</sup> W. W. Scott, "Standard Methods of Chemical Analysis," 4th ed., vol. I, p. 197. D. Van Nostrand Co., New York (1927).

<sup>27a</sup> G. C. Supplee and B. Bellis, *J. Dairy Sci.* 5, 455 (1922).

<sup>28</sup> C. S. King and G. Etzel, *Ind. Eng. Chem.* 19, 1004 (1927).

introduce appreciable amounts of copper. Copper-free distilled water should be used throughout.

As applied in 25 cc. volumes the error is 10–15 per cent on 0.005–0.018 mg. of copper and at 0.001–0.002 mg. becomes as great as 50 per cent.<sup>28a</sup> This definitely establishes a lower limit of its application. Opalescence has been reported to interfere if the solution is far from neutral<sup>28b</sup> although 3.8 has been reported as the optimum pH.<sup>28c</sup> At 0.02 mg. per final 25 cc. pH 7.0–8.5 is best although turbidity develops with time. With 5 mg. per 25 cc. no precipitate occurs at pH 3.0–3.5 but rapid precipitation occurs above 5.2. As a general condition pH 7.0 with 0.005–0.020 mg. of copper per final 25 cc. is probably best. Comparison should then be within 15 minutes.<sup>28a</sup>

**Sample—Inorganic.** Dissolve 5 grams of sample in 90 cc. of water. If insoluble in water dissolve in 1 : 1 nitric acid. Remove excess nitric acid by evaporating to dryness several times with concentrated hydrochloric acid. Take up with water and filter. Transfer to a 100 cc. volumetric flask and dilute to volume.

**Organic.** If the material is organic, decompose by adding 10 cc. of 10 per cent sodium hydroxide solution and 10 cc. of a saturated solution of sodium nitrate. Evaporate to dryness and ignite. Add hydrochloric acid and expel nitric acid as above. Transfer the aqueous solution of the sample to a flask of suitable size and dilute to volume.

**Organic Matter by Wet Digestion.**<sup>29</sup> Wet digestion is entirely trustworthy and avoids many possible sources of contamination and loss. Place the sample in a Kjeldahl flask. Close the flask with a funnel to prevent contamination. Add sufficient water to moisten the sample thoroughly and follow with 15 cc. of concentrated sulfuric acid. Heat until the material in the flask turns black and sulfur trioxide fumes are given off. Let cool. Add not more than 5 cc. of 20 per cent perchloric acid. The amount of sulfuric acid present when this is added must not be less than 5 cc. Add 2 cc. of fuming nitric acid. Heat with a small flame until no more oxides of nitrogen are given off. Increase the heat until sulfur trioxide fumes are evolved. Let cool. If the solution is not colorless repeat the addition

<sup>28a</sup> Lillian W. Conn, Arnold H. Johnson, H. A. Trebler and V. Karpenko, *Ind. Eng. Chem., Anal. Ed.* 7, 15-23 (1935).

<sup>28b</sup> L. H. Lampitt, E. B. Hughes, P. Bilham and C. H. F. Fuller, *Analyst* 51, 327 (1926).

<sup>28c</sup> D. L. Drabkin and C. S. Waggoner, *J. Biol. Chem.* 89, 51 (1930).

<sup>29</sup> Stefan Ansbacher, Roe E. Remington and F. Barlow Culp, *Ind. Eng. Chem., Anal. Ed.* 3, 314-20 (1931).

of perchloric acid and nitric acid and subsequent heating. The final volume will be less than the amount of sulfuric acid originally added as some is reduced to sulfur dioxide and water by the organic matter.

Wash into an Erlenmeyer flask and dilute with water until the sulfuric acid content is less than 15 per cent by volume. Heat to boiling and add a few drops of concentrated nitric acid. The nitric acid will decompose some of the hydrogen sulfide and give a sulfur precipitate to collect the copper sulfide and prevent a suspension of colloidal copper sulfide from forming.

Pass hydrogen sulfide through the solution until it is cold. Let the precipitate settle and then filter through an inorganic crucible with a porous bottom. Wash with 1 per cent acetic acid saturated with hydrogen sulfide until the washings give no test for iron. The copper sulfide must not be exposed to the air for any extended period of time. Place the crucible on a glass triangle over a glass crystallizing dish. Set this on a boiling water bath and add 1 cc. of fuming nitric acid to the crucible. When no liquid remains in the crucible wash with 2 cc. of water. Let stand on the water bath until the solution has evaporated to dryness. Place on a hot plate until no more acid fumes are given off. Avoid raising the temperature so high that decomposition of copper nitrate occurs. Add water to take up the residue. Transfer to a volumetric flask and dilute to a known volume according to the probable copper content indicated by the color.

This process requires no preliminary filtration from insoluble material and gives complete separation from 3rd-group metals and mercury salts. The final solution is neutral.

*Organic Matter by Dry Ashing.*<sup>29</sup> Heat the sample in a silica dish on a hot plate until it is charred. Place in an electric muffle at not over 400° for 30 minutes. Remove and let cool. Add 2 cc. of fuming nitric acid, or more if necessary to wet the ash, and evaporate to dryness on a hot plate. Organic matter may be completely oxidized at this point. If only a trace of organic matter remains, again add nitric acid and evaporate.

If considerable organic matter is left after the first evaporation with acid, replace in the muffle at 400° for 30 minutes. Remove, add nitric acid and repeat the evaporation as above. If necessary, repeat the ashing and evaporation with acid until carbon has been completely removed. The method is reliable if contamination with copper and losses due to high temperature of ashing are avoided.

Add 10 cc. of concentrated sulfuric acid to the ash and heat. Cool and add 25 cc. of water. Transfer to an Erlenmeyer flask with 35 cc. of water

and proceed as with wet ashing by heating to boiling, adding a few drops of concentrated nitric acid, etc.<sup>31</sup>

*Textiles.*<sup>32</sup> Put a 10 gram sample in a Kjeldahl flask with 2.5 cc. of fuming nitric acid, 2.5 grams of sodium sulfate and 2.5 grams of potassium sulfate. When reaction ceases add 13 cc. of concentrated sulfuric acid and complete the digestion. Dissolve the residue in water and add 1:1 ammonium hydroxide until just acid to Congo red. Add 5 cc. of 10 per phosphoric acid and dilute to 200 cc.

**Procedure—Duplication.** Place 10 cc. of 0.1 per cent solution of potassium ethyl xanthate in a Nessler tube of suitable size. Add an aliquot of the sample and dilute to 50 or 100 cc. The yellow color appears at once. Dilute in a similar tube 10 cc. of 0.1 per cent potassium ethyl xanthate nearly to volume, and add a standard copper sulfate solution containing 0.01 mg. of copper per cc. until the color is substantially duplicated. Cautiously add water and more standard to the duplicate until both color and volume are identical with the sample.

To confirm the correctness of this duplication prepare a series of 5 standards. One is to contain the amount of standard estimated by duplication. The others contain 0.05 and 0.10 cc. more and 0.05 and 0.10 cc. less of the standard than was estimated to be present by duplication. Get the final estimate of the sample from these standards.

*Dilution or Balancing.* To determine the results by dilution or balancing, dilute 10 cc. of the standard in a 500 cc. flask to about 400 cc., add 50 cc. of reagent and dilute to volume. The solution contains 0.002 mg. of copper per cc. Develop the color of the sample as for the duplication method and compare within 15 minutes.

Keep the xanthate reagent in an amber colored glass-stoppered bottle. Fresh xanthate solution should be prepared every few days.

**Standard**—Dissolve 0.3928 gram of pure copper sulfate, in water and dilute to 1 liter. Dilute 10 cc. of this solution to 100 cc. Each cc. contains 0.01 mg. of copper.

#### COPPER AS THE BROMIDE

The brown color developed by copper in solution as the bromide is

<sup>31</sup> See p. 154.

<sup>32</sup> Wm. C. Smith, *Proc. Am. Assoc. Textile Chem. Colorists*, 1930, 217-9; *Am. Dyestuff Repr.* 19, 583-5 (1930).

suitable for the determination of small amounts of copper present as sulfate.<sup>33</sup> Iron must be absent. The method is more sensitive than the ammonia method.

**Reagent**—Dissolve 5 grams of potassium bromide in 10 cc. of water. Cool with ice and slowly add from a buret 5 cc. of concentrated sulfuric acid with stirring, to keep the temperature from rising. Decant or filter through glass wool to remove potassium sulfate crystals. The reagent must be freshly prepared within a few hours of use.

**Procedure**—To the sample of 2 cc. add 0.5 cc. of concentrated sulfuric acid and 2 cc. of reagent. Compare with results obtained with 2 cc. of standard copper solution.

**Standard**—To 80 cc. of a suitable standard solution of copper sulfate add 20 cc. of concentrated sulfuric acid. Dilute with 20 per cent by volume sulfuric acid to the strengths desired.

#### COPPER BY POTASSIUM IODIDE

In the reaction of a copper salt with potassium iodide, iodine is set free. The time required for the liberated iodine to color a starch solution blue may be used for the estimation of traces of copper.<sup>34</sup> The theory of the reaction is discussed in detail in the original reference. The time for appearance of color is decreased with increasing concentrations of potassium iodide, and increased with increasing concentrations of starch and cuprous iodide. It is unaffected by slight acidity or by potassium sulfate, one of the products of the reaction. The time factor increases up to 30°, decreases from 30° to 68° and above the latter temperature no color is obtained in dilute solution.

The method compares favorably with other colorimetric methods on canned peas, shellac and other commercial products. It will detect 1 part in 5,000,000.

**Reagents**—Pure neutral potassium iodide crystals are obtained by fractional crystallization of the fused salt. Prepare a stock solution containing 150 grams per liter. Stir 0.5 gram of soluble starch to a thin paste with 50 cc. of cold distilled water. While stirring, add 100 cc. of

<sup>33</sup> G. Deniges and E. Simonot, *Bull. soc. pharm. Bordeaux* 54, 337-40 (1915).

<sup>34</sup> H. B. Dunncliff and K. Ram, *Kolloid Z.* 38, 168-70 (1926).



boiling water. Transfer to another container, washing out the first one twice with 10 cc. portions of water. Heat to boiling and boil exactly 1 minute. Dilute to 250 cc. This contains 2 mg. of starch per cc. Such a solution should be neutral and should be prepared a few hours before use.

The table given is to show relative, not absolute values, as the reagents used, especially starch, will vary according to source and treatment. By using the concentrations of reagents and standard as given the results will correspond to the first figure in column 9 of Table 3. These are the most concentrated solutions. By taking smaller volumes of the standard solutions and by suitable dilutions, values corresponding to those of the rest of the table may be obtained.

**Procedure**—Convert the copper in solution to sulfate by repeated evaporation to fumes with sulfuric acid. Place the sample solution in a 100 cc. Nessler tube and dilute to 80 cc. Mix 10 cc. of potassium iodide solution with 10 cc. of starch solution, for concentration see Table 3. Pour into the tube containing the sample and mix. Note with a stop watch the time interval in seconds before the appearance of a blue color. Compare with the table showing results with a series of standard copper sulfate solutions at a temperature of 16° to 18°. The time changes with temperature. A further aid to accuracy is to make the end of the time interval correspond to the appearance of an empirically established depth of color. The accompanying Table 3 gives the results obtained.<sup>34</sup>

**Standard**—From chemically pure copper sulfate crystals,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , recrystallized 3 times, prepare a standard solution containing 0.1 gram of copper per liter or 0.1 mg. per cc.<sup>35</sup>

#### COPPER BY POTASSIUM CYANIDE AND GUAIACUM

A very sensitive test for copper is by means of the blue color produced with potassium cyanide or potassium thiocyanate in the presence of a tincture of guaiacum resin.<sup>36,36a</sup> If the solution is acid the blue color appears only when the potassium cyanide has neutralized the acid. Excess cyanide is to be avoided as it changes the color to a brownish green. The sample must be protected from nitrous vapors, oxidizing agents such as

<sup>35</sup> See p. 150.

<sup>36</sup> H. Imbert, R. Imbert and P. Pilgrain, *Bull. soc. chim.* 35, 60 (1924).

<sup>36a</sup> R. Fleming, *Analyst* 49, 275 (1924).

TABLE 3  
COLORS DEVELOPED BY COPPER, POTASSIUM IODIDE STARCH  
Temperature 16-18°

Concentration of KI in grams per 100 cc.	1	2	3	4	5	6	7	8	9
	Time in sec.	Time in sec.	Time in sec.	Time in sec.	Time in sec.	Time in sec.	Time in sec.	Time in sec.	Time in sec.
0.5	0.01	0.01	0.02	0.005	0.01	0.02	0.005	0.01	0.02
0.005	0.01	0.01	0.02	0.005	0.01	0.02	0.005	0.01	0.02
Concentration of Starch in grams per 100 cc.	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
0.0010	120	105	105	0	0	0	0	0	0
0.0009	140	125	125	0	0	0	0	0	0
0.0008	160	140	143	0	0	0	0	0	0
0.0007	190	170	175	7.5	0	0	0	0	0
0.0006	235	220	225	13.5	5	6	0	0	0
0.0005	305	290	290	19	12	12	0	0	0
0.0004	385	375	375	24	17	19	0	0	0
0.0003	840-900	840	660-720	45	30	30	6	7	0
0.0002				105	73	75	15	15	11
0.0001				243	240	180-240	51	49	41
0.00005				660-720	660	660	151	151	132
0.00004					1020	1020	190	190	199
0.00003					1200	1200	240	240	240
0.00002					1440	1440	360-420	360-420	360-420
0.00001									
B - Blue									
P.B. - Pale Blue									
F.B. - Faintly Blue									
V.F.B. - Very Faintly Blue									

ferric salts, ammonia and ammonium salts, or if necessary these must be removed. Reagents must be especially pure.

**Sample—Legumes.** Digest about 1.9 grams of desiccated substances in a Kjeldahl flask with a 1:1 mixture of concentrated nitric and sulfuric acids. When decomposition is complete evaporate until no more sulfur trioxide fumes are produced. Take up with distilled water. A residue of calcium sulfate is left. To oxidize iron add a few drops of nitric acid. Precipitate iron and aluminum with a slight excess of 1:1 ammonium hydroxide, filter and render the filtrate just acid with a few drops of 10 per cent sulfuric acid. Evaporate to dryness and calcine slowly in the muffle furnace. When no more ammonia or sulfur trioxide vapors come off, take up the residue with distilled water and add a few drops of 0.1 N sulfuric acid to dissolve the copper oxide formed. Dilute to a known volume.

**Procedure—**Put 5 or 10 cc. of the cupric solution into a tube, add 3 or 4 drops of fresh tincture of guaiacum resin, then a few drops of a solution containing 1.50 grams of potassium cyanide per liter, until the blue color appears. If the mixture is too acid use a solution of 15 grams of potassium cyanide per liter. By comparing with suitable standards<sup>36b</sup> to which the same reagents have been added to the same volume of solution, determinations may be made at concentrations from 1 mg. to 100 mg. of copper per liter.

#### COPPER IN WATER IN THE PRESENCE OF LEAD

This procedure is given under lead.<sup>37</sup>

#### COPPER BY PYRIDINE AND THIOCYANATE

When copper is treated in aqueous solution with pyridine and an alkali thiocyanate, an insoluble compound,  $[\text{Cu}(\text{C}_5\text{H}_5\text{N})_2](\text{CNS})_2$ , is precipitated.<sup>38</sup> This compound is soluble in chloroform to give a green color, and when so dissolved is suitable for colorimetric estimation.<sup>39</sup> Bromobenzene is somewhat more satisfactory as the solvent.<sup>40</sup> With 0.01

<sup>36b</sup> See p. 145.

<sup>37</sup> See p. 193.

<sup>38</sup> G. Spacu, *Bul. soc. stiinte Cluj* 1, 352-5 (1922); *Analyst* 49, 275 (1924).

<sup>39</sup> R. Biazzo, *Ann. chim. applicata* 16, 96 (1926).

<sup>40</sup> Hans Kleinmann and Joachim Klinke, *Arch. path. Anat.* 275, 422 (1930).

mg. of copper the error was 5 per cent. and with 0.008 mg. it was 8 per cent. using a microchemical method.<sup>41</sup>

Manganese does not interfere. Ferric iron, if present, will give a brownish color. If necessary, copper is separated from the reduced iron solution by precipitation with hydrogen sulfide.<sup>42</sup> Ferrous and mercurous salts must be oxidized and nickel, cobalt and silver must be absent.<sup>43</sup> Interference of iron is also prevented by adding 1 cc. of 10 per cent tartaric acid and allowing the acidified solution to stand for a time before treatment with the reagents. Satisfactory results were obtained with less than 0.1 mg. of copper in the presence of 40 mg. of iron. With sample and standard similar in color 0.01–0.05 mg. may be estimated.<sup>23a</sup> The method has been found satisfactory<sup>44</sup> by other users.

In applying it to milk, large errors occurred unless the temperature of ashing was maintained reasonably low.<sup>45</sup> Chloroform was used for deflocculating the copper sulfide in separating it from iron. Phenolphthalein, introduced to obtain neutrality, is also a source of error.

**Sample—Organic Materials.**<sup>46</sup> Follow the methods of preparation by acid digestion or dry ashing given for the xanthate method.<sup>47</sup> Transfer the entire sample diluted to about 20 cc. or an aliquot diluted to that volume to a separatory funnel.

**Biological Materials.**<sup>42,41</sup> For blood use a 10 cc. sample. For liver or other organs select a suitable sample such as 5–7 grams. Ash the sample in a small Kjeldahl flask with a mixture of 10 cc. of concentrated sulfuric acid and 2 cc. of concentrated nitric acid. When decomposition is complete, let cool and dilute to 100 cc. with distilled water. Precipitate copper with hydrogen sulfide and filter.

Transfer the filter paper with the copper sulfide to a Pyrex test tube and decompose with 2 cc. of concentrated sulfuric acid. When decomposition is complete let cool and dilute to about 15 cc. Transfer to a small separatory funnel and make just alkaline to phenolphthalein with ammonium hydroxide. Render just acid with acetic acid and dilute to 20 cc. If the iron content is high add 1 cc. of 10 per cent tartaric acid.

<sup>41</sup> R. Schönheimer and F. Oshima, *Z. physiol. Chem.* 180, 249-52 (1929).

<sup>42</sup> C. A. Elvehjem and C. W. Lindow, *J. Biol. Chem.* 81, 435-43 (1929).

<sup>43</sup> Leslie J. Chalk, *Analyst* 55, 187-91 (1930).

<sup>44</sup> Ch. Benoit, *Ann. chim. anal. chim. appl.* 12, 66-9 (1930).

<sup>45</sup> H. T. Gebhardt and H. H. Sommer, *Ind. Eng. Chem., Anal. Ed.* 3, 24-6 (1931).

<sup>46</sup> Stefan Ansbacher, Roe E. Remington and F. Barlow Culp, *Ind. Eng. Chem., Anal. Ed.* 3, 314-20 (1931).

<sup>47</sup> See pp. 153-4.

*Biological Materials.—Alternative Method.*<sup>42</sup> Ash the sample at the lowest possible temperature in an electric furnace. Dissolve the ash in 10 cc. of concentrated hydrochloric acid and evaporate just to dryness. Take up the ash with 10 cc. of 1:3 hydrochloric acid by warming. Filter and wash. Evaporate the filtrate and washings to 10 cc. and transfer to a tube. Neutralize with 10 per cent sodium hydroxide solution and dilute to about 20 cc.

*Milk.*<sup>42</sup> Evaporate 250 cc. of milk to dryness and ash in a quartz dish at the lowest possible temperature. The large amounts of phosphates prevent direct extraction of the copper compound. Take up the ash with 25 cc. of 1:4 hydrochloric acid. Add 2 drops of concentrated nitric acid, heat to boiling and saturate with hydrogen sulfide. Let stand until cold and filter. Wash the filter with 50 cc. of 1 per cent acetic acid saturated with hydrogen sulfide.

Ash the paper with the precipitate. Dissolve the ash in 2 cc. of concentrated nitric acid and evaporate to dryness on a water bath. Take up the residue with 10 cc. of 1:3 hydrochloric acid by warming, and proceed as for the preceding sample.

*Bones.*<sup>42</sup> Use a modification of the procedure for milk.

*Wine.*<sup>53</sup> Select a volume of sample according to the probable copper content. Acidify and precipitate the copper as sulfide. Filter and dry the sulfide residue. Heat the residue in a covered crucible with concentrated nitric acid until oxides of nitrogen cease to be evolved. Remove the cover and evaporate to dryness. Acidify with 1:1 hydrochloric acid and again evaporate to dryness. Dissolve in water, add a few drops of acetic acid and dilute to about 20 cc. If the iron content is high add 1 cc. of 10 per cent tartaric acid before dilution.

*Aqueous humor.*<sup>53a</sup> Centrifuge the sample and pipet out a suitable volume of supernatant liquid. Since the copper content is only of the order of 0.14–0.18 mg. per liter this should be as large as possible. Add a few drops of concentrated sulfuric acid and then of concentrated nitric acid. Heat as usual for wet ashing. When ashing is complete dilute to a few cc. and neutralize with 1:1 ammonium hydroxide and use as sample.

**Procedure**—To sample and standard add 1 cc. of glacial acetic acid and 30 drops of pyridine. Add 3 cc. of 10 per cent potassium thiocyanate solution and 2 cc. of bromobenzene, accurately measured. Shake well

<sup>53</sup> J. Golsé, *Bull. soc. pharm. Bordeaux* 71, 24-30 (1933).

<sup>53a</sup> I. I. Nitzescu and I. Georgescu, *Compt. rend. soc. biol.* 117, 1135-7 (1934).

and let the bromobenzene settle. Draw off the green layer and compare with a standard similarly treated at the same time. This comparison is preferably made by the balancing method in a microcolorimeter. If necessary the determination can be repeated with a smaller sample or with more bromobenzene to give a color more easily estimated.

**Standards**—Prepare a solution of 0.1965 gram of copper sulfate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , in water and dilute to 1 liter. Each cc. contains 0.05 mg. of copper. For special purposes 10 cc. are diluted to 100 cc. to give a solution containing 0.005 mg. of copper per cc.

#### COPPER BY BENZIDINE

A quick comparison of a copper solution with a standard can be made by modification<sup>54</sup> of a qualitative method.<sup>55</sup> The color is stable for only 5 minutes. Copper must be present as the sulfate. Alkalies and alkaline earths may be present. Silver interferes. From 0.02 to 2 mg. may be estimated in a 100 cc. sample.

**Sample**—Prepare the sample as for the previous methods, free from organic matter. If heavy metals are present, render distinctly alkaline with ammonium hydroxide, boil for 5 minutes until only a faint odor of ammonia is present, and filter. Convert to the sulfate by evaporation until sulfur trioxide fumes nearly cease.

**Procedure**—To sample and standard diluted to about 70 cc. add 10 cc. of 3 per cent sodium salicylate solution, 10 cc. of concentrated ammonium hydroxide, 3 cc. of a 0.1 per cent solution of benzidine in 20 per cent acetic acid and 1 cc. of 1 per cent potassium hydroxide solution. Dilute each to 100 cc., mix and compare at once by balancing.

The color of the standard can be matched with an acidified solution of methyl orange as a permanent standard but such a standard is questionable at best.

**Standard**—As a suitable standard dissolve 0.1179 gram of copper sulfate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , in water and dilute to 1 liter. Dilute 100 cc. of this solution to 1 liter. Each cc. is equivalent to 0.003 mg. of copper. Suit-

<sup>54</sup> A. B. Shakhkeldian, *Zhur. Prikladnoi Khimii* 2, 475-82 (1929).

<sup>55</sup> N. A. and Iv. Tanahae, *J. Russian Phys.-Chem. Soc.* 60, 453 (1928); *Z. anorg. allgem. Chem.* 170, 113-27 (1928).

able amounts of this are to be selected, treated with the reagents at the same time as the sample and compared at once.

#### COPPER BY DIMETHYLGLYOXIME

A reddish violet color is produced when a chloride-free copper solution is treated with an oxidizing agent, silver nitrate and dimethylglyoxime (diacetyldioxime), followed by aqueous pyridine.<sup>56</sup> One part of copper can be detected in 10,000,000 parts of water. Not over 0.1 mg. per 100 cc. should be present in the sample. Ordinary distilled water will give a reaction by this method.<sup>57</sup> Small variations in the amount of pyridine present will radically affect the color. Excess pyridine causes fading. If the concentration of sulfuric acid is greater than 0.008 *N*, the coloration is faint and fugitive. Addition of more pyridine will not stabilize the color. Low sulfuric acid content gives erratic results. Buffers detract from the sensitivity of the reaction or cause too rapid fading. With less than 0.6 p.p.m. the color reaches a maximum in 5 minutes. Larger amounts reach their maxima and start to fade by that time.

Excess silver nitrate introduces a yellow color and promotes fading. Increase of ammonium persulfate promotes fading, probably due to acidity resulting from its decomposition. Sulfates and nitrates do not interfere. The presence of 2 mg. of sodium sulfate, magnesium sulfate, calcium sulfate or potassium nitrate per cc. does not affect the intensity or stability of the color. If the chloride content exceeds 0.0005 mg. per cc. the method is unreliable. More than 0.002 mg. of iron per cc. causes serious error. Cobalt present must not exceed 0.00002 mg. per cc.

**Procedure**—To 40 cc. of neutral sample, free from chlorides, add 1 cc. of 0.4 *N* sulfuric acid, 0.5 gram of ammonium persulfate, 0.5 cc. of a saturated solution of dimethylglyoxime in 95 per cent alcohol and 0.25 cc. of 0.5 per cent solution of silver nitrate. Mix well and add 1.5 cc. of a 10 per cent solution of pyridine in water. Mix well, dilute to 50 cc. and compare with a standard.

**Standards**—*Natural*. For a few samples, compare by balancing against a standard similarly treated at the same time as the sample.

*Artificial*.<sup>57</sup> Because of the fading of the natural standard, it is

<sup>56</sup> S. G. Clark and B. Jones, *Analyst* 54, 333-4 (1929).

<sup>57</sup> Loren C. Hurd and John C. Chambers, *Ind. Eng. Chem., Anal. Ed.* 4, 236-8 (1932).

to use artificial standards for any extended work. Prepare these from a potassium permanganate solution of suitable concentration, stabilized by addition of small amounts of potassium iodate and sulfuric acid. Standardize against natural standards.

The minus-green wedge of the Eastman Universal Colorimeter has also been used, as the color is almost identical with that developed with the reagent.

#### COPPER BY SODIUM DIETHYLDITHIOCARBAMATE

Sodium diethyldithiocarbamate by reaction gives the copper salt of diethyldithiocarbamic acid, which has a golden brown color.<sup>59</sup> This re-

is more sensitive than the older methods.<sup>60</sup> For increased sensitivity, the color can be extracted from aqueous solutions with carbon chloride<sup>61</sup> or with amyl alcohol<sup>59</sup> and may be read in the tintometer with a calibration curve. The method is more accurate than the muthate procedure<sup>63,64</sup> or that with pyridine and thiocyanate.<sup>28a</sup> A method by titration with chromotropic acid has been reported as more sensitive<sup>64a</sup> but that has been contradicted.<sup>28a</sup>

It will detect 1 part of copper in 100 million parts of distilled water. The most sensitive range is from 0.1 to 1.0 p.p.m. The color is not affected by alkalis. Cyanides interfere due to the presence of the complex ion. Most metals other than calcium and magnesium may interfere if present in sufficient amount. The color is stable for at least one hour. Cloudiness eventually develops due to oxidation of the reagent. The reagent will keep for several weeks in an amber bottle.

**Sample—Foods.**<sup>61</sup> Ash according to approved methods for the particular type of food. Dissolve the ash in 1:2 nitric acid. Add sulfuric acid in excess and evaporate to sulfur trioxide fumes. Dilute to a proper concentration and deposit the copper by electrolysis. Dissolve the cop-

---

D. McFarlane, *Biochem. J.* 26, 1022-33 (1932).

n, *Analyst* 54, 650-3 (1929).  
1345-51 (1930); L. A. Haddoe

vers, *Ana*

<sup>3</sup> Stefan

d. *Eng.*

*Anal. Ed.* 3, 314-20 (1931).

<sup>64</sup> W. Williams, *J. Dairy Research* 3, 93-100

<sup>64a</sup> Olive Sheets, Robert W. Pearson and Marvin Gieger,  
7, 109-10 (1935).



per from the electrode with a minimal amount of 1:1 nitric acid. Dilute to a standard volume and select a suitable aliquot for examination.

**Micro Samples—Blood.** For samples of 5 cc. or less, ash and then electrolyze in a micro apparatus with 2–3 volts and 15–30 milliamperes. Then dissolve in 1:3 nitric acid and proceed as for regular samples.

**Procedure**<sup>60</sup>—*Without Extraction or Stabilization.* Render the sample slightly alkaline with 1:1 ammonium hydroxide, or, if zinc is present, make it strongly ammoniacal. Add the sample to 10 cc. of a 0.1 per cent aqueous solution of sodium diethyldithiocarbamate, dilute to 50 cc. and mix. Compare with standards similarly treated.

*Stabilization Method.*<sup>67</sup> In some cases a turbidity obscures the result obtained. In that event mix 2 cc. of the unknown copper solution, 1 cc. of freshly filtered 0.1 per cent gelatine or gum tragacanth solution, 1 cc. of water and 0.6 cc. of 0.1 per cent aqueous solution of sodium diethyldithiocarbamate to give a volume of 4.6 cc. Increased accuracy in measurement is obtained by use of a micro buret rather than a calibrated pipet. Compare with a standard similarly treated.

*Extraction Method.*<sup>68</sup> If a 50 cc. sample contains less than 0.1 mg. of copper or if the solution contains ferric iron, extraction should be used.<sup>61</sup>

To the sample add 2 grams of citric acid. When this has dissolved add 1:1 ammonium hydroxide until the pH is not less than 9.0. This can be roughly estimated either by a very distinct odor of ammonia or by a very definite pink color with phenolphthalein on a spot plate. Dilute to about 70 cc. and add 10 cc. of 0.1 per cent diethyldithiocarbamate solution. Mix and extract with 4 successive portions of 2.5 cc. each of carbon tetrachloride. If more than a trace of color is shown by the final extraction, continue for further extractions. Dilute the carbon tetrachloride extracts to 20 cc. and compare the color with that from a known standard similarly extracted.

Alternatively, read the color in a Lovibond tintometer, using the yellow color only, and determine the copper content from a calibration curve. Results at 0.008–0.088 mg. of copper by this method agreed within 0.003 mg.

<sup>67</sup> Hal W. Moseley, Arthur G. Rohwer and Margaret C. Moore, *Science* 79, 507-8 (1934).

<sup>68</sup> L. A. Haddock and Norman Evers, *Analyst* 57, 495-9 (1932).

The extraction is also often carried out with amyl alcohol.<sup>70,71</sup> Difficulties due to mutual solubility of the normal alcohol are avoided by use of pure isoamyl alcohol, boiling range 129–31°, which is less soluble in water than the normal alcohol and therefore separates quickly and sharply.<sup>71</sup>

#### COPPER BY *m*-BENZAMINOSEMICARBAZIDE

A copper salt in aqueous solution reacts with *m*-benzaminosemicarbazide (eryogenine) to give a red color.<sup>72</sup> The reaction is fairly specific and sensitive. Concentrated copper solutions will give a precipitate. Neither color nor precipitate is given by sodium, potassium, caesium, magnesium, titanium, aluminum, lithium, lead, barium, tin, calcium or bismuth. Manganese and iron give a yellow color. Strontium, lead and mercury sulfates give light yellow precipitates. Lead carbonate gives a colorless precipitate. Nickel chloride and chromium and cobalt sulfate impart the colors of their ions to the solution. Zinc sulfate imparts a reddish-yellow color only after 5–6 hours. Silver nitrate is reduced to

The addition of lead acetate deepens the color developed by the reagent with copper. Magnesium, sodium and ferrous sulfates also intensify the color. Biuret and amines intensify and alter the red.

The reagent must be relatively fresh as it becomes yellow to brown in a few days. Acids or alkalis accelerate the decomposition and cause turbidity. Therefore the reaction should be carried out in nearly neutral solution. The maximum color intensity is attained in 30–50 minutes at 40°. The method will determine copper in amounts between 0.0005 and 0.02 mg. with an accuracy to 1.5 per cent. Lesser amounts give high results. Greater amounts alter the color toward yellow.

**Sample** <sup>73</sup>—*Blood*. Transfer 1 cc. of blood to a micro-Kjeldahl flask. Add 2 cc. of concentrated sulfuric acid and heat, first on a sand bath, then over a micro burner, until almost transparent. Let the slightly brown solution cool for a few seconds and add 5–10 drops of 30 per cent hydrogen peroxide. Heat until dense white fumes are evolved. Boil gently for 10–15 minutes, even if all color has disappeared. If necessary, add more hydrogen peroxide and repeat.

<sup>70</sup> L. Laget, *Ann. chim. anal. chim. appl.* 17, 145-7 (1935).

<sup>71</sup> R. W. Thatcher, *J. Am. Chem. Soc.* 55, 4524 (1933).

<sup>72</sup> Uichiro Sarata, *Japan J. Med. Sci. II. Biochem.* 2, 247-60 (1933)

Uichiro Sarata, *Japan J. Med. Sci. II. Biochem.* 2, 261-75 (1933).

To separate the copper, dilute to 30 cc. and transfer to a conical centrifuge tube. Add 0.5 cc. of 1 per cent magnesium chloride solution. Then add 10 *N* sodium hydroxide solution, drop by drop, until a turbidity persists. Add *N* sulfuric acid, drop by drop, to remove this turbidity. Add 2 cc. of *N* sulfuric acid in excess and mix. Pass washed hydrogen sulfide in through a fine capillary at the rate of 20–30 bubbles per minute for 40 minutes. Centrifuge for 20 minutes at 2500 r.p.m. Decant off the supernatant liquid and add 10 cc. of distilled water saturated with hydrogen sulfide. Stir the precipitate well with a glass rod to wash it, and centrifuge. Decant and wash twice more. Add 2 cc. of 1:2 nitric acid to dissolve the precipitate and heat in a boiling water bath for 15 minutes. Filter through a wet filter paper and wash well. Use this filtrate and washings as sample, or dilute to a known volume and pipet out a suitable aliquot.

**Reagent**—Dissolve 0.07 gram of “Cryogenine Lumière” in 100 cc. of distilled water by shaking at room temperature. Keep in a dark bottle and replace every second day.

Alternatively, prepare a 0.2 per cent solution of “Cryogenine Lumière” in distilled water. Store in a dark bottle in a cool place. It will keep at least 1 week. Dilute with 3 parts of distilled water before use.

**Procedure**—Use the sample solution or an aliquot containing 0.0005 to 0.03 mg. of copper. If not already acid, add 1:2 nitric acid until distinctly acid. Evaporate to dryness on a water bath in a glass dish. Heat for 15 minutes to remove all excess acid.

In 3 similar dishes place 1, 3 and 5 cc. of a standard solution containing 0.005 mg. of copper per cc. To each add 2 cc. of 1:2 nitric acid. Dilute to the same volume as the sample, evaporate to dryness and heat to remove excess acid.

To each standard and to the sample add 1 cc. of distilled water, 1 cc. of reagent and 1 cc. of pure lead acetate solution containing 0.1831 gram per liter. Mix by rotating the dishes. Cover and place in an incubator at 40° for 40 minutes. Compare the standard nearest in color to the sample, using a micro colorimeter.

**Standard**—Dissolve 0.5 gram of pure copper in 10 cc. of 1:1 nitric acid. Dilute the solution to 500 cc. Each cc. contains 1 mg. of copper. Dilute 5 cc. with water to 1 liter. Each cc. of this contains 0.005 mg. of copper as the nitrate.

ylthiocarbazono, called dithizone, gives colored precipitates with many heavy metals. These are soluble in organic solvents and, after suitable separations of interfering ions, may be compared colorimetrically. Dithizone and gold interfere. Over 50 mg. of ferric iron will interfere with the color of the reagent, but can be removed by extraction with ether extractable. This method applicable with accuracy only to 0.03-0.05 mg. has been modified<sup>75a</sup> to be applicable to 0.0005 mg. For that, great accuracy is necessary. Sodium pyrophosphate ammonium hydroxide of the original method be used for very minute amounts of the copper. It also removes the interference of tin. For accuracy, filtration of the precipitates must be avoided.

**Reagents—Dithizone Solution.** The reagent contains a yellow oxidation product and must therefore be purified. Dissolve it in carbon tetrachloride at the rate of 0.1 gram per 50 cc. Extract this solvent with 50 cc. of 1:100 ammonium hydroxide. Repeat this extraction. This dissolves the dithizone, but not the oxidation product. Combine the organic layers and acidify with 1:1 hydrochloric acid. Filter and wash with water and dry. Dissolve 0.01 gram carbon tetrachloride. Shake with 100 cc. of

tetrachloride until only a trace of pink color is extracted.

Acidify the aqueous layer with 1:1 hydrochloric acid and extract with three 30 cc. portions of carbon tetrachloride. Dilute the carbon tetrachloride extracts to 100 cc.

**Pyrophosphate-Carbonate Solution.** Dissolve 12 grams of recrystallized sodium pyrophosphate and 0.5 gram of sodium carbonate in water and dilute to 200 cc. Add about 50 cc. of dithizone solution in carbon tetrachloride to this in a separatory funnel and shake. Discard the carbon tetrachloride layer and shake the aqueous layer with successive 50 cc. portions of carbon tetrachloride until only a trace of pink color is extracted. Extract the aqueous layer with three 50 cc. portions of amyl

<sup>75</sup> Hellmut Fischer and Grete Leopoldi, *Angew. Chem.* 47, 90-2 (1934); *Wiss. Veröffentl. Siemens-Konzern* 12, 44-52 (1933).

<sup>75a</sup> R. M. Mehurin, *J. Assoc. Official Agr. Chem.* 18, 192-4 (1935).

alcohol to remove dithizone. Extract with two 50 cc. portions of carbon tetrachloride to remove amyl alcohol.

Carry this reagent through the procedure to be sure that not more than a trace of red color is given by it, if unsatisfactory repeat the purification. Use extreme precautions with the other reagents to be sure that they are not the cause of the contamination. Retest this solution every day or so to be sure it has not become contaminated from the glass.

**Sample—Organic.** Ash a suitable sample in a silica dish in a muffle at not over 500°. Dissolve the ash in 1:3 hydrochloric acid, by heating if necessary, and dilute to a known volume with water.

**Procedure—Tin Absent.** Pipet 10 cc. of sample containing not over 0.005 mg. of copper into a separatory funnel. Neutralize to methyl orange with 1:3 ammonium hydroxide. Add 2 drops of concentrated nitric acid to acidify. Add 1 cc. of the dithizone reagent and 2 cc. of carbon tetrachloride. Shake and draw off the carbon tetrachloride layer without any of the aqueous layer. Extract with 5–10 cc. of water containing 2–3 drops of concentrated hydrochloric acid. Separate the carbon tetrachloride layer quantitatively and wash again.

Add 5 cc. of the pyrophosphate-carbonate reagent and 5 cc. of water to the carbon tetrachloride layer and shake for 30 seconds. This removes excess dithizone. Separate and compare the color of the carbon tetrachloride layer with that of a series of standards similarly prepared containing 0.000, 0.0005, 0.001, 0.002, 0.003, 0.004 and 0.005 mg. of copper.

For larger amounts of copper, of the order of 0.05 mg. the extreme care in procedure may be relaxed, and the series of standards suitably altered.

**Tin Present.** With tin present, precipitation of stannous oxychloride may cause adsorption of copper when the sample is neutralized with ammonium hydroxide and then acidified. To avoid this add to the acid sample, before neutralizing, one-half volume of the pyrophosphate-carbonate reagent. Make distinctly acid with 1:1 hydrochloric acid. Neutralize to methyl orange with 1:3 ammonium hydroxide. Acidify with nitric acid as if tin were absent and proceed with the extractions.

#### COPPER BY DITHIOXAMIDE AFTER EXTRACTION WITH DIPHENYLTHIOCARBAZONE

Copper and lead can be extracted from alkaline solutions, free from

cyanides, with a dilute solution of diphenylthiocarbazone in chloroform.<sup>76</sup> After evaporation of chloroform the organic matter is destroyed with sulfuric acid. At this stage the solution should be free from iron. Any traces are rendered inactive by addition of citric acid.

The extract will be violet with a small amount of copper. If considerable lead or zinc is present it will be red. With large amounts of copper it is brown. Bismuth does not interfere with this method for copper, but does interfere with its application to estimation of lead. Dithiooxamide develops an olive-green color with copper. The average error is less than 1 per cent, the maximum 7 per cent.

**Sample—Medicinal Iron Preparations.** Mix a 2 gram sample, or more if the copper is under 20 p.p.m., in a 350 cc. flask with 5 cc. of water and 10 cc. of concentrated sulfuric acid. Heat gently and add 5 cc. of 30 per cent hydrogen peroxide to oxidize organic matter. Bring to a boil for 5 minutes and let cool slightly. Add 5 cc. more of 30 per cent hydrogen peroxide and again bring to a boil for 5 minutes. If necessary, add more hydrogen peroxide until oxidation is complete. About 15 cc. is normally required. Cool and add 5 cc. of water and 10 cc. of concentrated hydrochloric acid. Boil until clear. Cool and add a solution of 10 grams of citric acid in 50 cc. of water and 30 cc. of concentrated ammonium hydroxide. Again cool and add 1:2 ammonium hydroxide until neutral to litmus. Add 10 cc. of this ammonium hydroxide in excess and transfer to a separatory funnel. Extract with 3 successive 10 cc. portions of chloroform containing 0.1 gram of diphenylthiocarbazone per 100 cc. If an emulsion forms, disperse it with alcohol. Combine the extracts and wash 3 times with 50 cc. of water. The color of the wash water is due to excess reagent. Transfer to a small flask and evaporate the chloroform. Cool and add 0.5 cc. of concentrated sulfuric acid. Add a few drops of concentrated nitric acid and heat to destroy organic matter. If necessary, add a few more drops of nitric acid. Add a few drops of water to the solution after it cools and heat until white fumes are given off. This removes excess nitric acid.

The cooled residue contains the copper and lead present in the original sample. Add 10 cc. of water to dissolve the residue. Add 1 gram of citric acid and 4 grams of ammonium acetate. When solution is complete, add 1:1 ammonium hydroxide until alkaline to litmus and dilute to 100 cc.

<sup>76</sup>Noel L. Allport and G. H. Skrimshire, *Quart. J. Pharm. Pharmacol.* 5, 461-72 (1932).

*Foodstuffs.* Alter the preceding method of preparation of the sample as necessary in order to get complete solution of the required sample, and extract as above.

**Procedure**—Transfer 25 cc. of the sample solution to a 100 cc. Nessler tube. Add glacial acetic acid until approximately neutral to litmus. Add 2 cc. excess of glacial acetic acid and dilute to 100 cc. with water. Add 1 cc. of a solution of 0.1 gram of dithiooxamide in 95 per cent alcohol. An olive-green color develops which reaches its maximum in 1 minute.

Compare with a similar solution prepared from a suitable volume of standard copper solution containing 0.01 mg. of copper per cc.<sup>77</sup> Add 1 gram of ammonium acetate and 2 cc. of glacial acetic acid to the standard before development of color. This standard should not contain more than 0.06 mg. of copper in the final 100 cc. present after color development.

#### COPPER BY PIPERIDINIUM PIPERIDYLDITHIOFORMATE

When an alcoholic solution of piperidinium piperidyldithioformate is added to a solution of a copper salt, a very stable yellowish brown color is produced.<sup>78</sup> This is not altered by a slight excess of acid or alkali. Cadmium, mercury and bismuth do not interfere. Iron must be removed. This is provided for by a method of preparation of the sample which excludes iron, aluminum, chromium and mercury.

**Sample—Organic.** Place a large weighed sample in a silica dish on a hot plate until it is charred. Transfer to a muffle furnace at not over 400° and heat for 30 minutes. Remove and let cool. Add a few cc. of fuming nitric acid and evaporate to dryness on a hot plate. If only a small amount of organic matter now remains, moisten it with fuming nitric acid and again heat on the hot plate. For larger amounts, again heat in the muffle furnace for 30 minutes. After cooling, remove any small amounts of organic matter by heating with fuming nitric acid. Repeat if necessary. Do not heat above 400° in the muffle or low results may be obtained.

Dissolve the ash in 10 cc. of 1:1 hydrochloric acid. Dilute to 100 cc. and add 2 drops of concentrated nitric acid. Heat to boiling and pass

<sup>77</sup> See p. 155.

G. Harry, *Analyst* 56, 736-7 (1931).

a current of hydrogen sulfide through the solution until cold. Let the precipitate settle and filter through an inorganic filter. Wash the filter with 1 per cent acetic acid saturated with hydrogen sulfide until the washings no longer give a test for iron.

Place the crucible on a triangle over an evaporating dish. Add 5 cc. of fuming nitric acid to the crucible. When it has all gone through, wash the crucible with water. Heat the evaporating dish on a water bath until the water has evaporated. Then heat on a hot plate until excess acid is evaporated, avoiding decomposition of the nitrates. Dissolve the salts in 10 cc. of water and rinse into a suitable volumetric flask according to copper content. Dilute to volume.

**Procedure**—Transfer an aliquot of the copper solution to a 10 cc. tube. Dilute nearly to volume and add 1 cc. of a 0.1 per cent solution of piperidinium piperidyldithioformate in alcohol. Dilute to volume and compare with a standard <sup>78a</sup> similarly treated containing between 0.01 and 0.05 mg. of copper, according to the sample taken.

By calibration for a given dilution of sample, satisfactory results have also been reported with the Lovibond Tintometer.

#### COPPER BY $\beta$ -NAPHTHOL

Copper gives a color with  $\beta$ -naphthol, varying from yellow to blue.<sup>79</sup> While it has not been worked out as a quantitative method, it possesses the characteristics of one.

**Procedure**—Prepare a 0.02 per cent solution of  $\beta$ -naphthol in alcohol. Mix 2 cc. of this with 5 cc. of 6 *N* ammonium hydroxide solution. The color is pink. Add this to 5 cc. of unknown aqueous copper solution and compare with a series of standards similarly treated.

#### COPPER BY UROBILIN

In the presence of ammonia, urobilin gives a yellow to red color with copper.<sup>80</sup> The method is suitable for quantities of 0.01–0.0001 mg. Silver does not interfere. Mercury can be corrected for.

**Sample**—*Organic*. Ash according to methods approved for the par-

<sup>78a</sup> See p. 167.

<sup>79</sup> Charles C. Fulton, *Am. J. Pharm.* 105, 62-3 (1933).

<sup>80</sup> A. Emmerie, *Chem. Weekblad.* 27, 552-4 (1930).



ticular material. Dissolve the ash in 1:2 nitric acid. Add sulfuric acid in excess and evaporate to sulfur trioxide fumes. Dilute and deposit the copper electrolytically. Dissolve the copper in a minimal amount of 1:1 nitric acid. Add 1 drop of 0.1 *N* sulfuric acid and evaporate off the nitric acid. Dissolve in 1 cc. of distilled water.

**Procedure**—To the sample in a small vial add 1 drop of 10 per cent ammonium hydroxide. Add 0.4 cc. of a solution containing 5 mg. of urobilin per 100 cc. of redistilled alcohol. Dilute to 2 cc. The color develops at once. Compare with standards in a similar volume prepared from a copper sulfate solution containing 0.005 mg. of copper per cc.

If mercury is present add 1 drop of 1 per cent potassium iodide solution to the acid solution. A precipitate of mercuric iodide is formed. Dissolve it with 0.2 cc. of 1 per cent sodium thiosulfate solution before adding ammonia and the reagent. Add the same amounts of potassium iodide and sodium thiosulfate solutions to standards to be used for comparison.

**Standard**—Dissolve 0.3928 gram of pure copper sulfate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , in water and dilute to 1 liter. This standard contains 0.1 mg. of copper per cc. Dilute 5 cc. to 100 cc. This contains 0.005 mg. of copper per cc., as the sulfate.

#### COPPER BY COMPARISON OF THE COLOR OF ZINC MERCURITHIOCYANATE PRECIPITATES

Zinc gives a colorless precipitate with mercurithiocyanate, cobalt a blue one, copper a green one. When copper is precipitated in the presence of zinc the double precipitate,  $\text{ZnCu}[\text{Hg}(\text{CNS})_4]_2$ , colors the deposit a deep violet to black.<sup>82,82a</sup> For quantitative application the quantity of zinc must be closely standardized. Cobalt must be absent. The method is applicable to 0.001 to 0.050 mg. of copper with variations in the series of standards used.

**Reagents**—*Mercurithiocyanate Solution*. Dissolve 13.58 grams of mercuric chloride and 15.22 grams of ammonium thiocyanate in water and dilute to 1 liter.

<sup>82</sup> J. Golse, *Bull. soc. pharm. Bordeaux* 71, 16-24 (1933).

<sup>82a</sup> V. Cuvelier, *Z. anal. Chem.* 99, 15-8 (1934).

*Zinc Sulfate Solution.* Dissolve 14.40 grams of crystallized zinc sulfate in water and dilute to 1 liter.

*Zinc Reagent.* Mix 20 cc. of mercurithiocyanate solution and 50 cc. of zinc sulfate solution. Dilute to 1 liter. Shake, let settle and filter.

*Mercurithiocyanate Reagent.* Mix 100 cc. of mercurithiocyanate solution and 20 cc. of zinc sulfate solution. Dilute to 200 cc. and shake. Let settle and filter.

**Procedure**—Evaporate the sample to dryness in a glass tube. Let cool and add 1 cc. of zinc reagent. Stir until solution is complete. Add 1 cc. of mercurithiocyanate reagent. In mixing avoid rubbing the wall of the tube with the glass rod, which may cause the precipitate to stick to the sides. Pour into a fresh tube and wash the original tube with three 1 cc. portions of mercurithiocyanate reagent. Shake and let settle, or centrifuge. Compare the color of the precipitate with a series of standards similarly prepared containing 0.001 to 0.020 mg. of copper. For amounts to 0.05 mg., increase the amount of zinc reagent to 5 cc. and dilute with the washings to 15 cc.

#### COPPER BY SODIUM ARSENITE

When sodium arsenite and copper react the light green color of copper acid arsenite, Scheele's Green, is produced. The reagent prepared by dissolving anhydrous arsenious chloride in sodium hydroxide solution, is suitable. The color may be estimated colorimetrically.<sup>s2b</sup> A series of standards must be used as the color does not follow Beer's law.

The reagent will detect 0.8 p.p.m. of copper sulfate and is reliable up to 5 p.p.m. Above that concentration turbidity appears and nephelometric methods can be used. The color is permanent, and within the range of maximum accuracy, is not thrown down by centrifuging. Iron and magnesium interfere but are separated in the preparation of the sample.

**Sample—Tissue.** Digest 100 grams until organic matter is destroyed and isolate the copper by the sulfide method.<sup>s2c</sup> Dissolve the copper sulfide in 1 cc. of hot 1:1 nitric acid. Evaporate to dryness and convert to the oxide with a micro burner. Add 2 drops of 0.1 per cent sulfuric acid and heat to dissolve. Transfer to a graduated cylinder with water.

<sup>s2b</sup> Archibald N. Currie, *Biochem. J.* 18, 1224-6 (1924).

<sup>s2c</sup> See p. 153.

**Reagent**—Add 0.1 cc. of anhydrous arsenious chloride to 10 cc. of 10 per cent sodium hydroxide solution. The initial white precipitate disappears on mixing. Add another 0.1 cc. of the arsenious chloride and again mix. Add 10 cc. of water. The reagent does not deteriorate on standing in a stoppered bottle.

**Procedure**—Dilute the sample solution to 5 cc. Add 1 cc. of reagent and mix. Compare with a standard <sup>82d</sup> similarly treated at the same time.

### COPPER BY DIRECT GREEN B

A trace of copper alters the color of Direct Green B from green toward rose. This can be utilized for its estimation.<sup>82e</sup> The reaction occurs best at pH 6-9, distinctly acid media prevent the reaction. The best ratio of dye to copper is about 10:1. As little as 0.1 p.p.m. of copper can be detected. While the color is rapidly developed by heating, the same effect is obtained in 24 hours in the cold. The reaction is the same with sulfates, chlorides, nitrates and acetates of copper. Iron, chromium and mercury give a precipitate but no rose color. Silver gives a brownish-yellow color. Neutral salts such as potassium sulfate strongly retard the reaction. Colloids such as soap, gelatine and gum acacia in 1-10 per cent concentration mask the reaction. The method has been only roughly developed.

**Sample**—Neutralize to pH 7-8.

**Procedure**—Prepare 4 tubes containing 25 cc. of neutral sample and 0.1, 0.5, 2.5 and 10.0 cc. of 0.1 per cent solution of the dye. Mix and heat in a boiling water bath for 15 minutes. Cool and compare with results similarly obtained with known amounts of copper standard <sup>82f</sup> containing 1 mg. per cc., added to similar tubes of dye.

### COPPER BY REDUCED PHENOLPHTHALEIN

The qualitative method for the detection of copper by the leuco base of phenolphthalein red has been modified to a roughly quantitative

<sup>82d</sup> See p. 145.

<sup>82e</sup> W. P. Sisley and M. David, *Bull. soc. chim.* IV, 47, 1188-92 (1930).

<sup>82f</sup> See p. 145.

method.<sup>82</sup> The method is not specific as glycerine and several materials give it.

**Reagent**—Dissolve 2 grams of phenolphthalein and 20 grams of potassium hydroxide in 100 cc. of water. Add 10 grams of zinc dust and boil until completely decolorized. Filter and keep in a tightly stoppered bottle.

**Procedure**—To 10 cc. of sample add 4 drops of reagent. Mix and add 1 drop of 4–5 volume hydrogen peroxide. A rose to red color will develop in a variable time depending on the concentration of copper present. Compare with a series of standards<sup>82b</sup> containing 0.1–0.0001 mg. of copper per cc. A blank gives no color, even in several hours.

#### CADMIUM AS THE SULFIDE

Estimation of cadmium as the sulfide is somewhat more difficult than that of most of the sulfides because of the rather narrow pH range in which it can be completely precipitated.<sup>83,84</sup> Differences of less than 0.1 mg. per 50 cc. are indistinguishable in ordinary light but in the mercury arc can be read as low as 0.01 mg. per 50 cc. At 0.4–1.0 mg. of cadmium per 100 g. of organic sample the average error was 5 per cent.

The necessity of removing iron completely requires a long and complicated treatment of the sample. Lead must also be removed. The color deepens appreciably on standing. The standard must therefore be prepared at the same time as the sample.

**Sample—Organic.** Add sufficient nitric acid to cover organic material. Heat gently at first and when dissolved add 10 cc. of concentrated sulfuric acid. Continue to heat with occasional addition of small amounts of nitric acid until oxidation is complete. Heat until fumes of sulfur trioxide are given off freely. Dilute to 75 cc. and add 1.5 cc. of 0.1 per cent copper sulfate solution. Add 2 grams of sodium citrate. Neutralize with ammonium hydroxide and adjust with 1:1 hydrochloric acid to about pH 3.0 by the use of external indicators. Pass hydrogen sulfide into the solution for 5–10 minutes. Add 1 drop of 5 per cent aluminum chloride solution to prevent formation of colloidal cadmium sulfide and

<sup>82</sup> Pierre Thomas and Georges Carpentier, *Compt. rend.* 173, 1082-5 (1921).

<sup>82b</sup> See p. 145 for solution to use.

<sup>83</sup> Georg Hessel, *Biochem. Z.* 177, 146-55 (1926).

<sup>84</sup> Lawrence T. Fairhall and Leon Prodan, *J. Am. Chem. Soc.* 53, 1321-3 (1931).

let the suspension stand for 6–12 hours. Filter and wash. Dissolve the precipitate of cadmium sulfide in 2 cc. of *aqua regia* and evaporate to dryness. Dissolve in water again, add citrate and copper, adjust to pH 3.0 and precipitate as the sulfide. Repeat a third time, but omit the citrate and adjust to pH 2.0 with potassium hydroxide solution. Evaporate the next *aqua regia* solution of sulfide to dryness. Dissolve the cadmium chloride in water and dilute to a convenient volume.

**Procedure**—To an aliquot of the sample solution add 5 drops of 10 per cent potassium cyanide solution to prevent interference by copper. Dilute with water to 20 cc., add 5 cc. of a saturated solution of hydrogen sulfide and mix. Compare under a mercury arc with standards similarly prepared at the same time. If in doubt, let sample and standard stand a few hours and compare again.

**Standard**—Dissolve 1.6310 gram of anhydrous cadmium chloride or 1.9507 gram of cadmium chloride dihydrate in water and dilute to 1 liter. Each cc. contains 1 mg. of cadmium.

## CHAPTER XVII

### MERCURY

#### MERCURY AS THE COLLOIDAL SULFIDE

A TOXICOLOGICAL estimation of mercury<sup>1</sup> has been developed by comparing in the final solutions the brown color of mercuric sulfide. Arsenic, antimony, and mercury are precipitated as sulfides, and part or all of this mixed precipitate used. From it mercury is isolated and again converted to the sulfide.

**Sample—Organic.** For analysis of tissues or other organic matter grind a sample of 100–200 grams with water to a semi-liquid. Treat the finely divided material with potassium chlorate, using 10 per cent of the weight of sample taken, and concentrated hydrochloric acid, using 20 per cent of the weight of sample. Heat to destroy the organic matter, passing the gas evolved into water to absorb mercuric chloride. Filter and wash the residue on the filter with the liquid from absorption of mercuric chloride. Treat the filtrate with sulfurous acid to reduce excess potassium chlorate and arsenic compounds. Boil a few minutes to remove excess sulfurous acid. Pass a slow current of hydrogen sulfide through the solution for 18 hours. Separate the mixed sulfides by filtration.

To separate the mercury dissolve the mixed sulfides in *aqua regia* and evaporate to dryness *in vacuo*. Take up the residue with warm water and filter into a test tube. Dilute to about 20 cc., and add 1 cc. of concentrated hydrochloric acid. Add pure ammonium oxalate until the solution is saturated. Introduce into the solution a piece of pure copper<sup>2</sup> or gold<sup>3</sup> wire of such length that it is not quite covered by the solution. The purity of the copper wire if used, is of great importance, as copper often contains zinc, and this would distil off with the mercury, vitiating the determination. Electrolytic copper is best. It may be checked by heating in a glass tube to see whether any sublimate is given off, after which it should be ignited and reduced with methyl alcohol vapor. Heat

---

<sup>1</sup> C. Kohn-Abrest, *Ann. chim. anal. chim. appl.* 30, 353 (1925).

<sup>2</sup> A. Stock and W. Zimmermann, *Z. angew. Chem.* 41, 546-8 (1928).

<sup>3</sup> R. Thilenius and R. Winzer, *Z. angew. Chem.* 42, 287 (1929).

the upper part of the tube containing the solution and wire, in a flame, and draw it out until the two sections are connected by a rather narrow neck. Evacuate with a water pump and seal the tube by fusing the neck. This is to exclude air and avoid oxidation of the copper. Heat the sealed tube with its contents for 24 to 36 hours at 50 to 60°. Break the tube, remove the wire on which mercury has been deposited, and wash by placing the wire in several successive portions of fresh water, avoiding stirring. Dry over phosphorous pentoxide for 3 hours at room temperature.

A tube suitable for the distillation of the mercury from the wire is one of hard glass having a capillary constriction in the middle and drawn out to a fine capillary at the open end to reduce the movement of the air inside. Heat the closed end of the tube containing the wire for 5 minutes, cooling the second portion of the tube in running water, so that the sublimed mercury will be condensed on the walls. Do not permit the wire to fuse to the glass. The condensed mercury can be seen with a magnifying glass. Cut off the tube at the constriction.

*Alternative Method.*<sup>4</sup> In order to shorten the previous method, wash the precipitated and filtered mixed sulfides with a 10 per cent solution of potassium hydroxide. Dissolve the sulfides directly in 10 cc. of a solution containing 5 per cent of bromine and 10 per cent of potassium bromide. Decolorize this solution by addition of 10 per cent sodium bisulfite. Dilute to about 20 cc., neutralize with 1:1 hydrochloric acid and add 2 cc. in excess. Saturate the solution with pure ammonium oxalate and proceed as in the previous method to separate mercury on a copper spiral.

*Air.*<sup>5</sup> Absorb mercury vapors in air by washing it in chlorine water. Precipitate the mercury with a copper spiral and proceed as for the preceding method.

The alternative of removal of chlorine from such a solution is a problem. If air is passed through, some volatilization of mercuric chloride results.<sup>6</sup> Evaporation in a desiccator containing phosphorus pentoxide and soda lime is most satisfactory. If the solution is evaporated completely to dryness, some loss of mercuric chloride occurs. This may be due to hydrolysis. By evaporation to 0.05–0.5 cc. no loss of mercury occurs. This may be applied to a solution prepared by dissolving a

<sup>4</sup> J. Maingard, *Ann. méd. légale criminol. police sci.* 12, 14-8 (1932).

<sup>5</sup> E. V. Alekseevskii, *Zhurn. Prikladnoi Khim.* 4, 411-4 (1931).

<sup>6</sup> Vladimir Majer, *Z. anal. Chem.* 87, 352-6 (1932).

mercury deposit in chlorine water. By obtaining such a deposit, the interfering ammonia salts are separated.

**Procedure**—Treat the condensed mercury with iodine vapor to form red mercuric iodide. Dissolve the mercuric iodide in 1 cc. of a 1 per cent potassium iodide solution. Add 1 cc. of slightly ammoniacal 1 per cent gum arabic solution, and 1 cc. of clear saturated hydrogen sulfide solution. Stopper, mix, let stand 15 minutes and compare with a series of standards.

If the quantity of mercury is more than a few milligrams, dissolve the mercuric iodide from the sample in more than 1 cc. of potassium iodide solution and use an aliquot. As little as 0.0001 per cent mercury may be detected by this method. The colloidal sulfide solutions are claimed to be stable for an indefinite period.

**Standard**—As standard, dissolve 0.1354 gram of mercuric chloride in water and dilute to 100 cc. This corresponds to 1 mg. of mercury per cc. In test tubes take portions from 0.1 to 1.0 cc. of this standard with sufficient water to make 1 cc. in each case. To each add 1 cc. of 1 per cent potassium iodide solution, 1 cc. of ammoniacal 1 per cent gum arabic solution and 1 cc. of clear, saturated hydrogen sulfide solution.

#### MERCURY BY POTASSIUM DIPHENYLCARBAZONE

The blue color of mercury diphenylcarbazone<sup>7,8,9</sup> may be used for micro determination of 0.0005 to 0.01 mg. of mercury. If the semicarbazide is used as in some methods it is readily oxidized to the semicarbazone, even by air. The potassium derivative of diphenylcarbazone forms cerise to blue salts with zinc, lead, copper, mercury, iron, chromium, nickel and cobalt. It is therefore essential that the mercury be separated from other heavy metals before developing the color. Small amounts of strong electrolytes also interfere by causing deflocculation of the colloidal material.

**Sample**—Treat the sample as in a previous method<sup>10</sup> so that it is finally available as a deposit on the walls of a tube ending in fine open capillaries at both ends.<sup>3</sup>

<sup>7</sup> M. P. Cazeneuve, *Compt. rend.* 130, 1478 (1900).

<sup>8</sup> P. Ménière, *Compt. rend.* 146, 754 (1908).

<sup>9</sup> F. Feigl and F. Neuber, *Z. anal. Chem.* 62, 369 (1923).

<sup>10</sup> See p. 178.



Connect one end of the tube to a suction pump, and the other end to a source of chlorine gas dried by passing through sulfuric acid wash bottles. Pass chlorine through until the air has been substantially all replaced by chlorine. Fuse the tips of the capillaries. Put the micro-bomb now containing mercury and chlorine into a suitable holder and heat throughout the entire length at  $250^{\circ}$  for 15 minutes. The mercury is changed to mercuric chloride. Move the tube so that one of the capillaries projects from the holder. While cooling this part in water, heat the rest of the bomb for 3 hours at  $250\text{--}300^{\circ}$ . The mercuric chloride sublimes, and condenses in the exposed part. Let the tube cool, open the capillary containing no salt, connect to the suction pump and evacuate. Disconnect and allow to fill with air. Repeat the evacuation several times to remove all free chlorine. Heat the tip of the closed capillary so that the mercuric chloride is sublimed away from the extreme tip and cut this off.

**Reagent**—Boil 1 part by weight of diphenylcarbazide with 1 part of potassium hydroxide in 5 parts by weight of 95 per cent alcohol for 10 minutes. The reagent is a reddish orange solution.

**Procedure**—Dissolve the mercuric chloride in 2 cc. of water by sucking the water into the tube. Add 2 drops of a cold, saturated solution of urea<sup>6</sup> and one drop of alcoholic potassium phenylcarbazone solution as prepared. If necessary to correct for color of the reagent, use the Walpole type of comparator.<sup>13</sup> The color increases for 10–15 minutes, after which it is constant for several hours.

Yellow light is preferable for the comparison.<sup>2</sup> It is most stable in neutral solutions in which nearly 90 per cent of the color remained after 24 hours.<sup>3</sup> Disappearance is due to precipitation of the color, not to bleaching.

**Standard**—Prepare a standard mercuric chloride solution by dissolving 0.1354 gram in distilled water and diluting to 1 liter. Dilute 100 cc. of this solution to 1 liter and another portion of 10 cc. to 1 liter. These solutions contain 0.01 and 0.001 mg. of mercury per cc., respectively. By taking suitable portions of these prepare a series of 2 cc. standards containing 0.01, 0.008, 0.006, 0.004, 0.002, 0.001 and 0.0005 mg. of mercury and treat each with 2 drops of a cold saturated solution of urea and 1 drop of the reagent. Solutions containing 0.01 mg. mercury in 2 cc.

<sup>13</sup> See p. 11.

almost opaque with the reagent, those containing more than that must be diluted before the development of color.

### MERCURY B

When a dilute, nearly neutral solution of mercury is treated with an ioniacal solution of potassium iodide, a brown color is produced,<sup>16</sup> reversal of the Nessler method for ammonia. It has

has also been used to determine the amount of mercury in a high-frequency induction furnaces are in use. The diluted 1:2 with water, contained

the same

same time

The error invol

maximum accuracy. Unless the ratio of iodine 3:1 and 16:1 the reaction is not quantitative.<sup>18</sup> This limitation is a serious objection to the use of the reaction. Unless the solution is moderately alkaline no color develops. Excess ammonium chloride or the order of adding the reagents does not affect the results

**Sample—Fabric.** Heat a weighed sample of cloth to 50° with *aqua regia* diluted 1:2 with water. Pour off the extract and repeat the treatment about 6 times. Wash the residue with hot water. To avoid loss of material fit the flask with an air condenser. Concentrated *aqua regia* must not be used because of the volatility of mercuric chloride in hydrogen chloride. Make the combined extracts and washings slightly alkaline with sodium hydroxide solution and saturate with hydrogen sulfide. Let stand in a warm place, make just acid with acetic acid and let stand for a time.

Prepare a filter in a carbon tube by depositing a surface of kaolin, a on glass wool. Add dilute acid and wash well with boiling water. Use this for separating the precipitate and wash until all chlorides are removed. Treat the precipitate on the

<sup>16</sup> L. L. Lloyd and W. M. Gardner, *J. Soc. Chem. Ind.* 31, 10 (1912).

<sup>17</sup> L. Jordan and W. P. Barrows, *Ind. Eng. Chem.* 16, 899 (1924).

<sup>18</sup> E. H. Vogelenzang, *Pharm. Weekblad* 66, 65-7 (1929).

filter with warm 1:3 nitric acid and finally with boiling 1:3 nitric acid. The mercury remains on the filter as mercuric sulfide. Dissolve this with warm 1:2 *aqua regia* and neutralize with sodium hydroxide solution, or make alkaline in case of a very small quantity of mercury. Dilute to 10 cc.

**Procedure**—Prepare a standard solution to contain 0.1 mg. of mercury per cc.<sup>19</sup> and measure out 10 cc. of this standard. Add 5 cc. of the reagent to the sample and standard and compare the brown color. Prepare the reagent by dissolving 0.2 gram of potassium iodide, 6.0 grams of sodium hydroxide, and 2.0 grams of ammonium chloride in water. Dilute to 100 cc.

#### MERCURY BY PHOSPHOMOLYBDOTUNGSTIC ACID OR RELATED REAGENTS

Mercury separated as the element serves to reduce phosphotungstic, phosphomolybdic or phosphomolybdotungstic acid reagent such as is used for estimation of glucose, uric acid, phenol, etc.<sup>20</sup> The blue color produced is compared colorimetrically. It is sensitive to 2 p.p.m. of mercury.

**Sample**—Separate this on a metal wire and distil into a glass tube as previously described.<sup>21</sup> Seal one end of the tube.

**Reagent**<sup>22,23</sup>—Put 25 grams of molybdic oxide or 34 grams of ammonium molybdate in a large flask. Add 140 cc. of 10 per cent sodium hydroxide solution and 150 cc. of water. Boil 20 minutes to drive off ammonia fumes, even if molybdic oxide is used. Add 100 grams of sodium tungstate, 50 cc. of 85 per cent phosphoric acid and 100 cc. of concentrated hydrochloric acid. Dilute to 700–800 cc. and close the mouth of the flask with a funnel and a watch glass. Boil gently for 4 hours, adding water from time to time to replace that evaporated. Cool and dilute to 1 liter.

**Procedure**—Add 1 cc. of the reagent to the tube containing the mercury. Place the tube in a beaker of cold water and heat slowly. When

<sup>19</sup> See p. 181.

<sup>20</sup> V. Ciocalten and C. Titel, *Compt. rend. soc. biol.* 112, 621-2 (1933).

<sup>21</sup> See p. 178.

<sup>22</sup> Otto Folin and Hsien Wu, *J. Biol. Chem.* 38, 81-110 (1919).

<sup>23</sup> A phosphotungstic or molybdotungstic acid reagent can be substituted for the phosphomolybdotungstic acid reagent given here.

it reaches boiling, boil for 10 minutes. Let cool in the air. Mercury will have altered the color to yellowish green or even blue.

Transfer the contents of the tube to a test tube. Wash it out 4 times with 0.5 cc. portions of water. Add 1.5 cc. of 20 per cent lithium sulfate solution and 3 cc. of 20 per cent sodium carbonate solution. Shake well and add 1 more cc. of the sodium carbonate solution. Repeat this operation twice more so that 6 cc. of the sodium carbonate solution have been added. The yellow color of the reagent disappears in the alkaline medium.

Let the solution stand for 5 minutes and compare with a standard <sup>23a</sup> similarly treated at the same time.

#### MERCURY BY SELENIUM SULFIDE

Mercury vapor blackens a coating of selenium sulfide on paper or other suitable base,<sup>24</sup> the degree of blackening being a function of time of exposure, temperature, velocity of the air current over the reagent, and the concentration of mercury. Several elaborate types of apparatus for applying the method quantitatively have been developed.<sup>25,26</sup> In practical use the reagent is specific for mercury. Comparison<sup>25</sup> has shown that absorption of vapors by *aqua regia* spray or amalgamation of gold leaf gives only a small fraction of the amount present.

**Reagent**—To prepare active selenium sulfide saturate a 0.01 per cent solution of aluminium chloride with hydrogen sulfide at room temperature. Add normal selenious acid solution while continuing the current of hydrogen sulfide. Maintain an excess of the latter in order to insure precipitation of the yellow sulfide and to avoid formation of a colloidal solution. Filter the precipitate, wash and dry. Keep in a dark place.

Apply to paper by dipping a pad of cotton into the powder and rubbing lightly over the surface until a uniform film is formed. The paper should be smooth and dense and not too highly glazed.

**Procedure**—The gas to be analyzed for mercury is drawn into the apparatus by means of suction, the velocity being controlled by the speed of the motor driving the blower and measured by a flowmeter. The apparatus is made of Bakelite, to give better heat insulation and to make

---

<sup>23a</sup> See p. 181.

<sup>24</sup> B. W. Nordlander, U. S. Patent 1,711,742 (1929).

<sup>25</sup> B. W. Nordlander, *Ind. Eng. Chem.* 19, 521 (1927).

<sup>26</sup> Anon., *Gen. Elec. Rev.* 30, 442 (1927).

it possible to test corrosive gases such as flue gases.<sup>25</sup> The gas passes over an electric coil heater and into a tube ending in a nozzle. On issuing from the nozzle the gas impinges on a continuous strip of the coated paper which is drawn slowly by means of a clock motor past an opening in a diaphragm directly opposite the nozzle. A short time after exposure the color produced can be viewed from above and compared with a standard scale mounted beside the paper. In a portable form of the apparatus, the coated paper is fastened on a movable slide. After striking the paper the air is turned back into the annular space between the nozzle and the housing and is discharged through holes in the latter. In this space it passes over an adjustable thermoregulator which controls the heating current. Types of apparatus for both continuous and intermittent use have been designed.

The originator reports the detection of 1 part in 4 million by volume with a velocity of 1 meter of air per second, at 70°, after an exposure of 4 minutes.

By placing an incandescent lamp in front of the strip and a photoelectric cell behind it the amount of light reaching the cell will depend on the blackening of the paper. By using this to regulate a signal circuit, a bell is rung if the mercury concentration is dangerously high.

#### MERCURY NEPHELOMETRICALLY BY STRYCHNINE SULFATE AND POTASSIUM IODIDE

Mercury precipitated as the iodo-mercurate of an alkaloid can be estimated nephelometrically.<sup>29,30</sup> Strychnine is more satisfactory for this than quinine because it is less affected by acid. The precipitate is a complex,  $\text{HgI}_2 \cdot \text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2 \cdot \text{HI}$ . The sample should contain 0.002–0.01 mg. of mercury per cc. More dilute solutions down to 0.0002 mg. per cc. can be concentrated.

**Procedure**—To 1 cc. of sample solution containing 0.002–0.01 mg. of mercury as mercuric chloride add 0.1 cc. of 2.5 per cent strychnine sulfate solution. Add 0.5 cc. of 0.5 per cent potassium iodide solution, dilute to 2 cc. and mix. Compare with a series of standards similarly treated at the same time.

<sup>29</sup> G. Golse and M. Jean, *Bull. soc. pharm. Bordeaux* 69, 168-76 (1931).

<sup>30</sup> S. I. Sinyakova, *J. Gen. Chem.* (U. S. S. R.) 4, 1081-7 (1934).

## CHAPTER XVIII

### LEAD

#### LEAD AS THE SULFIDE

A small amount of lead in solution on the addition of hydrogen sulfide will not precipitate but gives a brown color of the colloidal sulfide. The amount of lead in the sample should be between 0.25 and 0.005 mg. The color produced is affected by electrolytes due to their effect on the size of the lead sulfide particles in colloidal suspension. A colloidal material such as gelatine will also affect the color produced. Copper should be absent or a special procedure used. One method to remove copper is by immersing metallic tin in the solution to be examined.<sup>1</sup> Copper has been estimated<sup>2</sup> to give a color which as compared with that of lead is as 1:0.813. Ferrous iron must also be absent from the final extract. The method is suitable for estimation of a trace of lead remaining in solution after electrodeposition of the major part.<sup>2a</sup>

**Sample—Baking Powder and Organic Samples.** Place 10 grams of sample in a Kjeldahl flask, washing down with a few cc. of water.<sup>3</sup> Add 200 cc. of concentrated sulfuric acid for samples containing not over 2 grams of starch, or 5 to 10 cc. of acid for each additional gram of starch. Add 5 to 10 grams of potassium bisulfate. Heat to boiling and digest until the carbonaceous matter is oxidized, adding a few cc. of nitric acid from time to time to hasten oxidation of carbon. Let cool, dilute and evaporate in a casserole to complete removal of sulfur trioxide fumes.

To remove copper, iron, aluminum and similar metals extract the residue 5 or 6 times with portions of a solution containing 25 cc. of 5 per cent sulfuric acid and 5 cc. of 95 per cent alcohol. Digest the undissolved residue containing lead sulfate with 50 cc. of hot ammoniacal ammonium acetate solution. Extract 2 or 3 times, filter through the same paper that was used for the removal of iron, and wash with water. The am-

---

<sup>1</sup> J. C. Thresh, *Analyst* 49, 125 (1924).

<sup>2</sup> C. Pyriki, *Z. anal. Chem.* 64, 326 (1924).

<sup>2a</sup> Merle Randall and Marian N. Sarquis, *Ind. Eng. Chem., Anal. Ed.* 7, 2-3 (1935).

<sup>3</sup> W. W. Scott, *Chem. News* 131, 18 (1925).

moniacal ammonium acetate solution consists of concentrated ammonium hydroxide neutralized with glacial acetic acid, after which 5 per cent by volume of concentrated ammonium hydroxide is added.

Evaporate the extract to about 40 cc. Cool and pour into a 100 cc. Nessler tube, adding the washings.

*Tissue.*<sup>4</sup> Weigh 2 grams of dry tissue into a 50 cc. Kjeldahl flask. Add 3 cc. of sulfuric acid. Add concentrated nitric acid, drop by drop, as long as there is any reaction. Let stand over night. Add 1 cc. of 30 per cent hydrogen peroxide and heat cautiously until the organic matter is destroyed and a colorless solution obtained. If necessary, let cool and add a further portion of hydrogen peroxide.

Dilute the solution to about 25 cc. with water and transfer quantitatively to a centrifuge tube with a conical bottom. Centrifuge. Decant and wash the residue with 2 cc. of 1 per cent sulfuric acid. Again centrifuge and decant.

Add 2 cc. of 70 per cent ammonium acetate solution to the residue. Heat in a boiling water bath for 10 minutes. Centrifuge and decant the clear solution containing the lead into a 25 cc. flask. Repeat the digestion with ammonium acetate 4 times. Repeat once with water. Use the combined extracts as sample, diluting to a known volume and aliquoting if necessary.

*Organic Samples.*<sup>5</sup> Destroy the organic matter in a suitable sample by wet ashing. Dilute with water and heat to sulfur trioxide fumes to remove nitric acid. Cool, dilute with water to about 100 cc. and add 2 grams of citric acid. Add 1:1 ammonium hydroxide until alkaline, and 1 cc. of 10 per cent potassium cyanide solution. Transfer the cold solution to a separatory funnel and extract with 10 cc. of a 0.1 per cent solution of diphenylthiocarbazone in chloroform. Remove the chloroform layer and extract with two further 5 cc. portions. The reagent solution, which was red, changes to a deep green due to the lead compound formed. This compound is insoluble in water but soluble in chloroform. If the third extract is bright red, repeat the extraction once more. Combine the chloroform extracts and wash with 25 cc. portions of water. A red color in these is due to excess reagent and does not represent a loss of lead.

Transfer the combined chloroform solutions to a flask and evaporate the chloroform on a water bath. Cool and add 0.5 cc. of sulfuric acid to the residue. Add concentrated nitric acid, drop by drop, and heat to destroy organic matter. When the organic matter has been removed,

<sup>4</sup> Ludwig Pincussen and Ernst Brück, *Biochem. Z.* 265, 58-60 (1933).

<sup>5</sup> Noel L. Allport and G. H. Skrimshire, *Analyst* 57, 440-9 (1932).

dissolve in 10 cc. of water. Add 2 grams of ammonium acetate and 1:1 ammonium hydroxide until the solution is distinctly alkaline

is not provided for.

*Organic Samples. Alternative Method.*<sup>6</sup> Decompose the sample with mixed sulfuric and nitric acids. Dilute with water and heat to sulfur trioxide fumes. The volume should be about 1 cc. Dilute to 5 cc. and add about 0.25 gram of finely divided calcium sulfate to assist in collection of the lead sulfate precipitate. Filter on an inorganic filter and wash with 5 cc. of 1 per cent sulfuric acid

ion<sup>7</sup> and use this solution as  
di

gum acacia or gel

extraction given

as above.

*ic Copper.* Place 5  
50 cc. of 1:1  
hydrochloric

H. Cheftel and J. Blass, *Compt. rend.* 195, 146-7 (1932); *Bull.*

Baking Powder and Organic Samples, starting with the

1, 5,

(1932).

<sup>9</sup> See p. 170.



cm. of clean copper filings retained on a 30 mesh sieve. Place another cotton plug 1 cm. thick on top, leaving the remaining space for wash water. Suck through the tube 10 cc. of a mixture of equal parts of 1:1 hydrochloric acid and 1:1 nitric acid. Wash with water.

Allow the solution being tested to percolate through this tube several times in 2 hours.<sup>11</sup> Wash 4-5 times with cold water. Transfer the contents of the tube to a small beaker. Cover with 10 cc. of glacial acetic acid and let stand for 15 minutes. Decant from the copper and wash twice with water. Drain as completely as possible and give a final washing. Add 12 drops of sulfuric acid and 3 cc. of concentrated nitric acid to destroy organic matter. Evaporate to fumes, add 2 cc. more of nitric acid and repeat the evaporation. Cool and take up with 10 cc. of ammonium acetate solution made by mixing 1 volume of 1:1 ammonium hydroxide with 2 volumes of 35 per cent acetic acid. Use this solution for the determination.

*Copper.* Various feasible methods for separation of lead from copper have received detailed investigation.<sup>11a</sup> The following was preferred.

Wash a 50 gram sample with hot concentrated hydrochloric acid and then with distilled water. Dissolve in 200 cc. of concentrated nitric acid. Boil off the brown fumes and add 1200 cc. of water. Add 350 cc. of concentrated ammonium hydroxide and 2 cc. of 50 per cent lead-free disodium phosphate. Add 50 cc. of an ammonium carbonate solution prepared by passing carbon dioxide into 1:2 ammonium hydroxide until a precipitate starts to form. Mix well and add 25 cc. of 20 per cent lead-free calcium chloride solution with stirring. Let stand over night and filter on an inorganic filter. Dissolve the precipitate in 25 cc. of concentrated hydrochloric acid. Neutralize with 1:1 ammonium hydroxide and slightly acidify with concentrated hydrochloric acid. Saturate with hydrogen sulfide and filter. Wash on the filter with 1:50 hydrochloric acid saturated with hydrogen sulfide. Dissolve the sulfides in a suitable volume of concentrated nitric acid. Evaporate to crystals on a water bath. Cool and take up with 10 cc. of ammonium acetate solution made by mixing 1 volume of 1:1 ammonium hydroxide with 2 volumes of 35 per cent acetic acid solution. Dilute to a suitable volume and use all or an aliquot as sample.

*Commercial Phosphoric Acid.* Weigh from 100 to 400 grams of commercial phosphoric acid, depending on its concentration and purity, into

<sup>11</sup> B. S. Evans and S. G. Clarke, *Analyst* 51, 224-9 (1926).

<sup>11a</sup> Bartholow Park and E. J. Lewis, *Ind. Eng. Chem., Anal. Ed.* 7, 182-3 (1935).

a liter flask and dilute with water.<sup>12</sup> Warm if necessary to make the solution clear, cool and dilute to 1 liter. Transfer an aliquot containing 5 to 15 grams of acid to a 250 cc. beaker. Add 10 cc. of 25 per cent sulfuric acid and about 1 gram of a paste of calcium sulfate. Add sufficient 95 per cent alcohol to make a 70 per cent solution. When the precipitate begins to settle, filter and wash with 70 per cent alcohol. Transfer the filter paper and precipitate containing the lead to a small beaker and add 50 cc. of ammoniacal ammonium acetate solution made by pouring 1 volume of 1:1 ammonium hydroxide into 2 volumes of 35 per cent acetic acid. Boil for a few minutes and decant through a filter into a 200 cc. flask. Digest the residue with 2 fresh portions of ammonium acetate solution. The greater part of the calcium sulfate should have been dissolved. Cool the combined filtrates, acidify with acetic acid, dilute to 200 cc. and transfer a 50 cc. aliquot to a Nessler tube. Determine the lead as sulfide by the procedure which follows. If the nature of the color of the unknown differs from that of the standard, prepare a new sample, igniting the precipitated calcium and lead sulfates below redness before digesting with ammonium acetate solution.

*Calcium Phosphate.*<sup>13</sup> Dissolve a 1 gram sample in 10–15 cc. of 2 N hydrochloric acid. Add 30 cc. of 50 per cent ammonium citrate solution. Add sufficient 1:1 ammonium hydroxide to render the solution distinctly alkaline and add 2 cc. of 2 per cent potassium cyanide solution.

*Urine.*<sup>14</sup> Place 500 cc. of the sample in a 7 inch evaporating dish. Add 50 cc. of concentrated nitric acid and evaporate. From time to time add more urine until 1000 cc. has been evaporated to about 30 cc. Transfer the residue to a 9 cm. silica dish, using 10 cc. of concentrated nitric acid, and some distilled water if necessary, to transfer the residue. Evaporate to dryness. Place in a muffle furnace and heat until fuming ceases and the mass is charred. Heat cautiously to prevent ignition. Gradually push further into the furnace until ashing is complete at 450–500°. This usually takes about 30 minutes. All nitrates must be decomposed.

Cool and add hot water until the dish is approximately 65 per cent filled. Heat on a hot plate to boiling and break up lumps with a glass rod. Carefully add 1:2 hydrochloric acid until the solution is neutral or slightly acid, then 2 cc. in excess. The proper excess of acid is important.

<sup>12</sup> W. H. Ross, C. B. Durgin and R. M. Jones, *J. Ind. Eng. Chem.* 14, 534 (1922).

<sup>13</sup> John R. Nicholls, *Analyst* 56, 594-5 (1931).

<sup>14</sup> R. W. Tannahill, *Med. J. Australia* 1, 195-201 (1929).

Filter on lead-free paper, collecting the filtrate in a 300 cc. Erlenmeyer flask. Cool and dilute to 250 cc. Pass in hydrogen sulfide for 1 hour and let settle over night. Filter and wash 3 times with water saturated with hydrogen sulfide. Dissolve the sulfides with 2 cc. of hot 1:1 nitric acid and wash with 2 cc. more of the same acid, collecting the solution in the flask used for precipitation. Wash 6 times with hot distilled water and evaporate to about 20 cc. Transfer to a silica beaker and evaporate to 5 cc. Add 1 cc. of concentrated sulfuric acid and heat to copious fumes. Cool and add 20 cc. of cold water and 10 cc. of 95 per cent alcohol. Mix, let stand over night and filter. Wash with 33 per cent alcohol containing 3 per cent of sulfuric acid.

Dissolve lead sulfate from the paper with 5 cc. of hot ammonium acetate solution.<sup>15</sup> Add another 5 cc. of the ammonium acetate solution and wash 6 times with hot water. Collect the filtrate in a 50 cc. Nessler tube as sample.

*Urine. Very Low Lead Content.*<sup>16</sup> This treatment of sample was developed for comparison of the lead content of urine of city dwellers with that of country residents, in a study of the effect of "ethyl" gasoline.

Prepare nitrosyl sulfuric acid by passing sulfur dioxide into cold concentrated nitric acid until it is saturated. Add 80 cc. of it to 1 liter of urine in small portions, mixing well during the addition. Evaporate to one-third the original volume. Add a few drops of amyl alcohol from time to time to prevent foaming. Add 20 cc. of concentrated nitric acid and evaporate until charring begins. Let cool, add more concentrated nitric acid and repeat the evaporation until the solution is clear. Evaporate to sulfur trioxide fumes to decompose all nitrosyl sulfuric acid and continue to heat until separation of calcium salts occurs. Before the mass solidifies, dilute with cold, then hot water to 400 cc. Filter to remove silicic acid. Ash the precipitate with nitric and sulfuric acids. Evaporate to sulfur trioxide fumes. Transfer to a platinum dish and treat with hydrofluoric and sulfuric acids to volatilize silica. This recovers any absorbed lead. Dilute the acid solution and add to the solution of the original sample.

Add 5 cc. of 10 per cent citric acid solution and 4 cc. of a 1 per cent copper nitrate solution. Prepare an indicator solution containing 1 gram of methyl orange and 1.4 gram of xylene cyanol FF in 500 cc. of 50 per cent alcohol. Add 5 drops of this indicator. Add concentrated ammonium

---

<sup>15</sup> See p. 186.

<sup>16</sup> A. G. Francis, C. O. Harvey and J. L. Buchan, *Analyst* 54, 725-35 (1929).

hydroxide until the solution passes through a neutral grey color and reaches a green representing a pH of about 4.5.

Saturate with hydrogen sulfide and filter. Destroy the paper by treatment with the minimum possible amount of nitric and sulfuric acids and evaporate to sulfur trioxide fumes. Add 10 cc. of water and neutralize with concentrated ammonium hydroxide. Add 15 cc. of water and 1 cc. of concentrated nitric acid.

Electrolyze with a cylindrical gauze anode rotated at 1500–2000 r.p.m. using 70–80°, 1.5–2.0 volts and 0.3–0.4 amperes. After 1 hour remove and wash the anode. Dissolve the deposit of lead dioxide in 5 cc. of 1:1 nitric acid and 1 cc. of 95 per cent alcohol. Transfer to a silica beaker and add 1 cc. of concentrated sulfuric acid. Evaporate to fumes and cool. Add 10 cc. of cold water and 5 cc. of 95 per cent alcohol. Mix well and let stand over night. Filter and wash with 33 per cent alcohol containing 3 per cent of sulfuric acid.

Dissolve the lead sulfate from the paper with 2 cc. of hot ammonium acetate solution<sup>17</sup> and wash the paper well with hot water. Collect the filtrate in a tube of suitable size according to the lead content and dilute if necessary.

*Solutions High in Iron.*<sup>18</sup> With less than 100 p.p.m. of lead present, iron can be removed by extraction as the thiocyanate with organic solvents. At 12°, lead thiocyanate is practically insoluble in the extraction medium. The method is applicable to various solutions obtained from the preceding samples. It does not provide for interference by bismuth.

Add 5 cc. of 1:5 nitric acid to the sample of 30 cc. of solution in a separatory funnel. Add 5 cc. of a saturated aqueous solution of ammonium thiocyanate and mix. Add 15 cc. of amyl alcohol and 15 cc. of ether. Cool to 12° and shake. Draw off the aqueous layer and extract with fresh organic solvent. Use the aqueous layer as sample after aliquoting if necessary.

*Water and Aqueous Solutions.* Evaporate 5 liters to 300–400 cc., add 10 to 15 cc. of a 10 per cent solution of aluminum sulfate, 5 to 10 cc. of dilute sulfuric acid and 25 cc. of concentrated ammonium hydroxide.<sup>19</sup> Heat to boiling, allow the precipitate to settle until almost cold, and filter. Treat the precipitate as for organic samples, beginning with extraction of this residue with sulfuric acid and alcohol.<sup>20</sup>

<sup>17</sup> See p. 186.

<sup>18</sup> J. Hubert Hamence, *Analyst* 57, 622-6 (1932).

<sup>19</sup> W. W. Scott, *Chem. News* 131, 19 (1925).

<sup>20</sup> See p. 186.

*Water. Alternative Method.*<sup>21</sup> Add 1 cc. of 1 per cent gum arabic solution and 0.5 cc. of 30 per cent acetic acid to 100 cc. of sample. Mix well and use as sample. Make the same additions to the standards used for comparison.

**Procedure**—If copper is suspected, add 1 cc. of a 10 per cent solution of potassium cyanide. Add 1 cc. of a strong colorless solution of ammonium sulfide or 10 per cent sodium sulfide or saturated hydrogen sulfide solution. Dilute to a suitable volume and compare with standards containing the same reagents as the sample.

**Standards—Balancing.** Dry finely ground lead nitrate at 100°. Dissolve 0.1598 gram in distilled water and dilute to 1 liter. Each cc. corresponds to 0.1 mg. of lead. As an alternative, weigh 0.1831 gram of lead acetate, dissolve in distilled water and dilute to 1 liter.

Prepare a series of standards by adding 0.5, 1, 2, 5 and 10 cc. of the standard lead solution to suitable portions of the ammoniacal ammonium acetate, ammonium citrate, or other electrolyte solution used with the sample. Add 1 cc. of potassium cyanide solution, if used with the sample, 1 cc. of sulfide solution and dilute to the same volume as the sample. Permanent standards may be prepared to match the lead standards by combining varying amounts of solutions of the sulfates of cobalt, ferric iron and copper.<sup>22</sup>

*Natural Standard by Duplication.*<sup>21</sup> Mix the same reagents as are present in the sample and dilute to nearly the same volume. Add a lead solution containing 0.02 mg. of lead per cc.<sup>24</sup> until a color check is obtained. Adjust the volume of the standard to match the sample.

*Artificial Standard by Duplication.* Grind 0.25 gram of humic acid with 2.5 cc. of 10 per cent sodium hydroxide solution and disperse in 300 cc. of cold distilled water. Filter and dilute the filtrate to 1 liter. Standardize against colors developed from a standard lead salt. Add this standard solution from a buret to water to duplicate the color developed in the sample.

## LEAD AS THE SULFIDE IN WATER

The problem of estimation of lead in water has had much detailed

<sup>21</sup> O. Liebknecht and L. Gerb, *Angew. Chem.* 45, 744-5 (1932).

<sup>22</sup> See p. 67.

<sup>24</sup> Dilute 20 cc. of the standard above to 100 cc.

study,<sup>24a</sup> and requires separate discussion. Various sources of error in addition to copper are iron, organic coloring matter, aluminum, etc. Concentration of lead on calcium carbonate is a method of separation from very dilute solutions.

**Procedure**—*Less Than 10 mg of Iron per Liter and No Organic Color.* Measure 80 cc. into a tube. Add 0.4 cc. of 4 *N* hydrochloric acid and 4 drops of 10 per cent potassium cyanide solution. Add 10 cc. of 20 per cent sodium potassium tartrate solution and 10 cc. of a 20 per cent solution of ammonium chloride in 2 *N* ammonium hydroxide. Mix. Prepare a similar standard from 80 cc. of lead-free distilled water with the same reagents and a known amount of standard lead solution. Use the standard prepared from lead nitrate for the sulfide method. To the standard add 2 drops of a sulfide reagent prepared by dissolving 10 grams of hydrated sodium sulfide,  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ , in 25 cc. of water and diluting to 100 cc. with glycerol. Mix and compare the standard containing the reagent with the sample without reagent as a check on the natural color of the solution. Then add 2 drops of reagent to the sample, mix and compare sample and standard within 10 minutes after they were mixed. The reading of the sample is therefore corrected for the natural color of the sample without the sulfide reagent.

*Less Than 10 mg. of Iron per Liter but with Yellow Organic Color.* Mix 100 cc. of sample with 0.5 cc. of 4 *N* hydrochloric acid and 5 cc. of 5 per cent ammonium persulfate solution. Evaporate to 50 cc. and cool to 50°. Add 4 drops of 10 per cent potassium cyanide solution and 10 cc. of 20 per cent sodium potassium tartrate solution. Mix well and filter if turbid, washing the filter with 0.1 *N* hydrochloric acid. Add 10 cc. of a 20 per cent solution of ammonium chloride in 2 *N* ammonium hydroxide. Mix and compare with a standard of known lead content to which the reagent has been added, to estimate the natural color of the sample. Add 2 drops of sulfide reagent to the sample, mix and compare with the standard within 10 minutes after the reagent was added to the standard.

*Low Lead Content.* Mix 1 liter of water or more, containing 0.05-0.1 mg. of lead, with 5 cc. of 4 *N* hydrochloric acid. Add 5 cc. of 10 per cent potassium cyanide solution and 3 drops of 1 per cent phenolphthalein solution. At the same time run a parallel blank with lead-free distilled

---

<sup>24a</sup> J. F. Reith and J. de Beus, *Chem. Weekblad* 32, 205-10 (1935).

water. To each, while stirring, add 4 *N* sodium hydroxide solution to definite alkalinity. Add 0.5 gram of precipitated calcium carbonate to each and stir occasionally for 1 hour. Let stand for several hours, decant the clear layer and filter sample and blank on 5 cm. Büchner funnels. Wash each with 25 cc. of water rendered alkaline to phenolphthalein. Discard the filtrate and washings. Dissolve the precipitate from each filter with 50 cc. of boiling 0.4 *N* hydrochloric acid. Wash the filters with about 50 cc. of hot water containing a trace of hydrochloric acid. To the filtrates add 5 cc. of 5 per cent ammonium persulfate solution and evaporate to 80–90 cc. in 20 minutes. Add 0.1 gram of hydrazine hydrochloride to each and evaporate to 70–80 cc. in 10 minutes. Cool and compare the sample and blank with a developed standard. This contains standard lead solution, 2.5 cc. of 4 *N* hydrochloric acid, 10 cc. of a solution containing 15 per cent ammonium chloride and 20 per cent ammonium acetate solution. Dilute this standard to 100 cc. and add 2 drops of the sulfide reagent. After reading the undeveloped sample and blank add 10 cc. of the ammonium chloride-ammonium acetate solution to sample and blank, dilute to 100 cc., add 2 drops of sulfide reagent, mix and compare with the standard within 10 minutes after adding the sulfide reagent.

To confirm the supposition that the color is that of lead, add 10 cc. of 4 *N* hydrochloric acid and the color should disappear.

*Copper and lead present.* To determine both copper and lead in water add to the concentrated sample 10 cc. of a 2 per cent solution of potassium bicarbonate and 1 to 3 drops of a 1 per cent solution of potassium ferrocyanide.<sup>25</sup> Dilute to 100 cc. and compare with distilled water containing the same reagents by adding a standard copper sulfate solution in which 1 cc. contains 0.1 mg. of copper, until the colors match in the same volume of solution.

*Lead in the presence of copper.* To determine lead add to each 2 to 3 drops of a 10 per cent solution of potassium cyanide, 2 cc. of concentrated ammonium hydroxide and 2 to 3 drops of 10 per cent sodium sulfide solution. The solution containing copper only is decolorized and the one containing both copper and lead now has only the brown color from the lead sulfide. To the colorless solution add a standard lead solution to match the color of the sample.

The value for copper and lead less the value for lead, times 0.813 gives the correct value for copper. Difficulty with ferric iron can be eliminated

<sup>25</sup> L. W. Winkler, *Z. angew. Chem.* 26, 38 (1913).

by addition of phosphoric acid<sup>26</sup> or by its reduction with hydrazine sulfate.<sup>27</sup>

### LEAD IN URINE AS THE SULFIDE

By use of a special procedure below lead may be estimated in urine without ashing.

**Procedure**<sup>28</sup>—Heat 100 cc. nearly to boiling and add *N* sodium carbonate solution until the liquid becomes turbid and a precipitate forms on gentle agitation. This usually requires about 1 cc. Let settle and decant through a filter paper. Wash with distilled water made just with carbonate. Dissolve the precipitate in 2 cc. of glacial acetic acid and 5 cc. of water. Wash and add 0.5 cc. of a solution of 10% lead acetate. Dilute to 24 cc., add 1 cc. of 10% potassium cyanide solution.

causing a finer dispersion.

### LEAD IN CREAM OF TARTAR AS THE SULFIDE

**ure**<sup>29</sup>—]

per cent potassium cyanide  
and 4 Ccc. of water by volume  
per cent sodium sulfide  
of tartar to which known amounts of lead  
and add 0.5 cc. of 10% lead acetate  
s from lead-free cream  
been added.

### LEAD AS THE CHROMATE

may be  
presence of zinc  
by this  
ion with  
identity produced by 0.1% lead acetate  
for

, Z. . 88, 325-36 (1932); 29,

2).

<sup>27</sup> J. A. Wiegand, *Chem. Weekblad* 30, 262-3 (1933).

<sup>28</sup> J. C. Thresh, *Analyst* 49, 127 (1924).

<sup>29</sup> E. L. Andrews, *Analyst* 49, 129 (1924).

<sup>30</sup> L. S. Van der Vlugt, *Chem. Weekblad* 25, 194 (1928).

<sup>31</sup> P. W. Dankwortt and E. Jurgens, *Arch. Pharm.* 266, 374-82 (1928).



detectable change. Sodium acetate and copper do not affect the turbidity. Large amounts of iron are undesirable. Alkaline earths must be absent.

**Procedure—Zinc absent.** Evaporate the lead solution to dryness with a drop of strong nitric acid. Take up with water, filter, and transfer the filtrate to a comparison tube. Add 1 drop of *N* acetic acid and 1 drop of 10 per cent potassium chromate solution. Dilute to 100 cc., mix and compare the turbidity with that produced in suspensions containing from 0.03 to 0.60 mg. of lead per 100 cc.

**Zinc present.** In the presence of zinc evaporate the extract without nitric acid, filter and transfer the filtrate to a comparison tube. Add 1 cc. of zinc acetate solution containing 20 mg. of zinc per cc. and dilute to 100 cc. Shake, add 5 drops of a 5 per cent solution of potassium chromate and compare after 10 minutes with standards containing 3 cc. of 10 per cent acetic acid, varying amounts of a lead acetate solution containing 0.1 mg. of lead per cc., and other reagents as above.

**Standard Solution—**Dissolve 0.1831 gram of lead acetate,  $(\text{H}_3\text{COO})_2\text{Pb} \cdot 3\text{H}_2\text{O}$ , in water and dilute to 1 liter. Each cc. corresponds to 0.1 mg. of lead.

#### LEAD AFTER PRECIPITATION AS THE CHROMATE

By this procedure the lead is precipitated as the chromate, filtered and dissolved in strong acid. The lead is estimated from the yellow color of the chromate.<sup>32</sup> The method is not extremely sensitive.

**Procedure—**To 10 cc. of solution of lead, free from tin, phosphate or other interfering substances, add 30 cc. of 5 per cent potassium nitrate solution, 5 drops of glacial acetic acid and 1 cc. of 10 per cent potassium bichromate solution. Boil for 5 minutes, allow to stand over night and filter on an inorganic filter. Wash with 5 per cent potassium nitrate solution and dissolve in 30 cc. of 1:1 nitric acid. If the acid is colored, boil to remove oxides of nitrogen before use.

The solution of bichromate in a Nessler tube is matched by addition to another tube of 30 cc. of 1:1 nitric acid and sufficient standard potassium bichromate solution to match the color.

<sup>32</sup> B. S. Evans, *Analyst* 53, 267-75 (1928).

**Standard**—Prepare the standard potassium bichromate solution to contain 0.71 gram per liter. Each cc. will then be equivalent to 1 mg. of lead.

#### LEAD BY *s*-DIPHENYLCARBAZIDE

By addition of excess potassium bichromate, lead is quantitatively precipitated. As an indirect method for lead the amount of excess potassium bichromate is estimated from the pink to violet color produced with *s*-diphenylcarbazide.<sup>33-35</sup> A definite color is obtained with 0.1 p.p.m. of chromium.

**Sample**—Select the sample and dissolve by preceding methods. Precipitate lead as the sulfide and filter. Wash with 5 per cent sodium sulfide solution and dissolve in boiling 1:3 nitric acid. Evaporate to dryness and heat at about 150° for 1 hour. When cool dissolve the lead nitrate in 10 cc. of water and again evaporate to dryness. Dissolve in 4 cc. of water and add 1 cc. of 1 per cent sodium acetate solution. This converts any residual nitric acid to acetic acid.

**Procedure**—Add the resulting lead solution to 25 cc. of a solution of potassium bichromate containing 0.1420 gram per liter. Each cc. of this solution is equivalent to 0.2 mg. of lead. If the bichromate solution is added to the lead solution, a basic chromate of variable composition is precipitated. Add about 0.1 gram of finely divided acid- and alkali-washed asbestos, shake for 10 minutes and filter into a 100 cc. volumetric flask. Wash well and dilute to the mark.

Test 5 cc. of the solution with 2 drops of 1:3 nitric acid and 2 drops of diphenylcarbazide solution to insure the presence of excess bichromate. If a pink to violet color is not obtained excess bichromate is absent. In that case evaporate 50 cc. of filtrate to dryness, take up with 5 cc. of water and add to another 25 cc. portion of bichromate solution.

For development of color for comparison, add to 25 cc. of the solution 10 drops of 1:3 nitric acid and 5 drops of a 4 per cent solution of diphenylcarbazide in glacial acetic acid. Compare by balancing or dilution against a standard containing 12.5 cc. of potassium bichromate

<sup>33</sup> A. Cazeneuve, *Analyst* 25, 331 (1900).

<sup>34</sup> A. Moulin, *Bull. soc. chim.* 31, 295 (1904).

<sup>35</sup> P. Breteau and P. Fleury, *J. pharm. chim.* 10, 265 (1914).

<sup>36</sup> B. S. Evans, *Analyst* 46, 285 (1921).

solution, 12.5 cc. of water and the same reagents as above. This standard requires modification if the colors are not similar in intensity.

**Calculation**—Consider each cc. of bichromate solution added as equivalent to 0.2 mg. of lead, allowing for any aliquots taken. Consider the bichromate added as standard in the same way and by difference estimate the lead in the sample. If, for example, the sample is one gram and 25 cc. of bichromate solution are used, the equivalent as lead is 5 mg. If the solution used balances at 20 mm. with a standard of 12.5 cc. at 25 mm. the residual bichromate amounts to 3.125 mg. of lead and 1.875 mg. of lead are present in the sample, or 0.001875 per cent.

#### LEAD AS THE CHROMATE BY *s*-DIPHENYLCARBAZIDE

The high degree of insolubility of lead chromate permits its precipitation, filtration and estimation of lead from the combined chromate radical.<sup>38,39</sup> The method by estimation with *s*-diphenylcarbazide is applicable to 0.01–0.2 mg. of lead. Aluminum, arsenic, barium, bismuth, cadmium, calcium, cobalt, copper, iron, magnesium, manganese, mercury, nickel, silver and tin do not interfere.

The results of cooperative tests have not been particularly good. The method is apparently sound, but requires refinement as to details.<sup>40</sup> It is reported to be more lengthy and less accurate than titration of the resulting bichromate solution after liberation of the equivalent as iodine.<sup>40a</sup>

**Sample**—Wet-ash the sample and dilute the solution of ash to 250 cc. Add 25 per cent sodium hydroxide solution until the solution is faintly alkaline to methyl orange. Make just acid with 1:2 hydrochloric acid. At this stage the sample solution should be clear. Dilute to about 300 cc. and saturate with hydrogen sulfide. Continue the passage of hydrogen sulfide for 1 hour, keeping the inlet tube near the bottom of the beaker. Cover the beaker with a watch glass and let it stand overnight. Filter the upper clear layer through an 11 cm. filter paper and wash the precipitate onto the paper with hot water. Wash the filter with about 5 cc. of a mixture of 3 volumes of strong ammonium sulfide solution and 2 volumes of 1:1 ammonium hydroxide. Wash the filter thoroughly

<sup>38</sup> Edward W. Krasn and J. B. Ficklen, *J. Ind. Hyg.* 13, 140-3 (1931).

<sup>39</sup> B. Jones, *Analyst* 55, 318-20 (1930).

<sup>40</sup> Wm. J. McCarthy, *J. Assoc. Official Agr. Chem.* 15, 370-3 (1932).

<sup>40a</sup> C. N. Myers, Florence Gustafson and Binford Throne, *J. Lab. Clin. Med.* 20, 648-57 (1935).

with hot water. Discard the filtrate and washings. Dissolve the lead sulfide from the filter with 5 cc. of hot 1:1 nitric acid, added in several portions, using a 250 cc. beaker as receiver. Wash the original beaker used for the sulfide precipitation with 5 cc. more of hot 1:1 nitric acid and pour it through the filter. Wash this beaker once with hot water and then wash the filter with hot water until it is free of acid.

Remove hydrogen sulfide from the acid solution of lead by boiling. When cool, add a drop of phenolphthalein solution as indicator. Add 25 per cent sodium hydroxide solution until the solution is alkaline. Add 2 cc. of 50 per cent acetic acid and 5 cc. of 0.1 *N* potassium bichromate solution. Mix well, cover with a watch glass and boil for 2 minutes. Let stand for 3 hours, or, preferably over night at room temperature. Filter on a small paper. Wash the beaker and filter very thoroughly with hot water. All soluble chromates must be removed from both. Discard the filtrate and washings. Rinse the beaker in which precipitation was carried out and dissolve the precipitate from the paper with 25 cc. of 1:20 hydrochloric acid. Repeat with a fresh portion of acid. Wash the paper thoroughly with cold water. Collect the solution and washings in a 250 cc. volumetric flask.

**Procedure**—Add 2 cc. of a 1 per cent solution of *s*-diphenylcarbazide<sup>41</sup> in glacial acetic acid, dilute to volume and mix.

Compare the pink color with standards prepared from a solution containing 0.0142 gram of potassium bichromate per liter. Each cc. of this standard is equivalent to 0.02 mg. of lead.

#### LEAD BY TETRAMETHYLDIAMINODIPHENYLMETHANE

Small amounts of lead converted to the form of lead dioxide may be used for oxidation of tetramethyldiaminodiphenylmethane to a blue diphenylmethane dye.<sup>42-45</sup> From the intensity of this color the lead content is estimated. The method has been particularly developed for biological work but should have other applications. It will detect 0.005 mg. of lead.

<sup>41</sup> See p. 625.

<sup>42</sup> M. A. Trillat, *Compt. rend.* 136, 1205-7 (1903).

<sup>43</sup> M. Klostermann, *Naturwissenschaften* 14, 1116-8 (1926).

<sup>44</sup> A. Seiser, A. Necke and H. Muller, *Arch. Hyg.* 99, 158-64 (1928).

<sup>45</sup> A. Necke, P. Schmidt and M. Klostermann, *Deut. med. Wochschr.* 52, 1855-6 (1926).

**Sample**—The sample may be of dried feces, urine, tissue, blood or other organic materials. The weight will vary according to the source. Decompose according to known methods with fuming nitric and sulfuric acids.<sup>46</sup> When decomposition of organic matter is complete evaporate to dryness. Take up with about 50 cc. of water and add 1:3 ammonium hydroxide until faintly ammoniacal. If sufficient iron is not present to act as an indicator *p*-nitrophenol may be used. Add about 0.1 gram of finely divided aluminum or iron powder. Pass hydrogen sulfide through the solution for 1 hour to displace air, then treat with hydrogen sulfide under pressure for 24 hours. This is conveniently done in some form of gas wash-bottle. All lead compounds will be precipitated.

Filter through a very fine mat in a Gooch crucible or on a Witt plate. Wash the precipitate on the filter with 3 per cent potassium cyanide solution to remove any copper or its compounds. As a safety precaution empty this cyanide solution from the suction flask before proceeding.

Add 3 cc. of concentrated sulfuric acid to 100 cc. of 50 per cent alcohol and pass hydrogen sulfide through the solution for 1 hour. Wash the precipitate with this to remove iron, aluminum, manganese and calcium. Traces of iron and aluminum may be left without interfering. Lead remains behind as lead sulfide or sulfate.

Dissolve the lead sulfide from the filter with 5 cc. of fuming nitric acid and evaporate just to dryness. Dissolve the lead sulfate from the filter with 5 cc. of 5 per cent sodium acetate solution, add to the dried residue from the lead nitrate solution and heat if necessary until solution is complete. Adjust to neutrality to litmus paper by addition of small amounts of either 1:10 sulfuric acid or 1:10 ammonium hydroxide.

Place on a boiling water bath and add saturated bromine water until a permanent yellow color is obtained. Sodium hypochlorite or sodium persulfate<sup>47</sup> may also be used for oxidation. Hydrogen peroxide will not oxidize the lead. Heat for 2 hours. Lead will be precipitated as lead dioxide. Filter while hot on the same Gooch crucible from which the lead nitrate and lead sulfate were dissolved, first washing the crucible by filtering a few cc. of bromine-water to avoid the presence of reducing agents. Wash thoroughly to remove traces of residual bromine which will also oxidize the reagent.

<sup>46</sup> For details of methods of destruction of organic material refer to pp. 496-9.

<sup>47</sup> A. D. Petrov, *J. Russ. Phys. Chem. Soc.* 60, 311-6 (1928).

**Procedure**—Prepare a 1.0 per cent solution of tetramethyldiamine-diphenylmethane in glacial acetic acid. Dissolve the lead dioxide by filtering 25 cc. of this solution through the crucible, collecting the filtrate separately from all previous filtrates. The glacial acetic acid used as solvent for the reagent dissolves the lead dioxide and the latter oxidizes the reagent to give a blue color. In dissolving lead dioxide in the reagent, the latter should be added in small portions and each completely washed through before adding the next portion. The last portions filtered should come through the crucible colorless, indicating that solution of lead oxide is complete.

Compare with a series of standards prepared by the action of known amounts of lead dioxide on the reagent. The dilution method may also be used.

#### LEAD BY DIPHENYLTHIOCARBAZONE (DITHIZONE)

Benzeneazothioformic acid phenylhydrazine, or diphenylthiocarbazone, gives colored precipitates in alkaline solution with copper, silver, gold, zinc, cadmium, mercury, thallium, tin, lead, manganese, iron, nickel, cobalt, ruthenium, rhodium, palladium, osmium, iridium and platinum. These precipitates are readily soluble in organic solvents such as chloroform, carbon tetrachloride, etc.<sup>48</sup> From this there has been developed a colorimetric method for estimation of lead.<sup>49-50</sup> Results agree well with those by the electrolytic method.<sup>51</sup> The reagent is referred to as dithizone. The method has also been modified into a titrametric procedure.<sup>52</sup>

The green color of the reagent turns to red when the lead compound is formed. The procedure developed will determine 0.05 per cent of lead in zinc, cadmium, copper, mercury, silver, arsenic, tin, aluminum, beryllium, etc. Stannous tin and thallous ion must be oxidized. Other metals are prevented from interfering by addition of potassium cyanide to the sample. Interference by oxidizing agents can be prevented by addition of 0.01–0.2 gram of hydroxylamine. Bismuth interferes but can be removed from the carbon tetrachloride extract with a solution of potassium

<sup>48</sup> Hellmut Fischer, *Wiss. Veröffentlich. Siemens-Konzern* 4, 158 (1925); *chemie* 8, 319 (1930); *Z. angew. Chem.* 42, 1025-7 (1929); *Ibid.* 46, 442 (1933).

<sup>49</sup> Hellmut Fischer and Grete Leopoldi, *Wiss. Veröffentlich. Siemens-Konzern* 12, 44-52 (1933); *Angew. Chem.* 47, 90-2 (1934).

<sup>50</sup> John R. Ross and Colin C. Lucas, *Can. Med. Assoc. J.* 29, 649-50 (1933).

<sup>51</sup> H. J. Wichmann, *J. Assoc. Official Agr. Chem.* 18, 182-9 (1935).

<sup>52</sup> E. S. Wilkins, Jr., C. E. Willoughby, E. O. Kraemer and F. L. Smith, 2nd, *Ind. Eng. Chem., Anal. Ed.* 7, 33-6 (1935).

cyanide. As an alternative extract bismuth from a nitric acid solution adjusted to pH 2.0, using the reagent. Under those conditions bismuth reacts and is extracted by the reagent but lead does not react. From 0.002 to 0.200 mg. of lead may thus be determined in the presence of 0.500 mg. of bismuth.<sup>53</sup>

The method has been applied to spray residues from apples with an accuracy within 5 per cent, using the reading as lead dithizone.<sup>53a</sup>

**Sample—Urine.**<sup>50</sup> Add 3 cc. of concentrated ammonium hydroxide and 1 cc. of a saturated solution of monoammonium phosphate to 100 cc. of urine. Mix well and centrifuge to remove the mixed phosphates in which the lead is completely entrained. Decant the supernatant liquid. Add *N* hydrochloric acid until the precipitate is completely dissolved, adding water if necessary to produce a convenient volume. Heat to boiling and saturate the solution with hydrogen sulfide. After standing, add a small amount of paper pulp and centrifuge to remove the sulfide. Dissolve the precipitated sulfide in 10 cc. of *N* hydrochloric acid. Filter and wash the filter. To the filtrate add *N* ammonium hydroxide until it is alkaline to bromothymol blue. Use this solution as the sample.

**Tissue.** Ash in a muffle furnace at not over 450°. Dissolve the ash by the usual procedures and continue as for urine above.

**Spinal Fluid.** Proceed as for urine except for the preliminary addition of 0.2 cc. of 5 per cent calcium chloride solution for each 10 cc.

**Bones, Organs and Body Fluids.**<sup>53b</sup> Ash the dried material with sulfuric and nitric acids, neutralize and dilute to such an extent as necessary and precipitate the sulfide. Proceed as for urine. Wet oxidation is preferable to dry ashing.<sup>53c</sup>

**Metals.**<sup>48</sup> Dissolve in a suitable acid according to size and type of metal and use the acid solution, suitably diluted, as sample.

**Reagent—**Use as prepared for copper.<sup>54</sup>

**Procedure—Comparison as Dithizone.** The sample or aliquot used

<sup>53</sup> C. E. Willoughby, E. S. Wilkins, Jr., and E. O. Kraemer, *Ind. Eng. Chem., Anal. Ed.* 7, 285-6 (1935).

<sup>53a</sup> O. B. Winter, Helen M. Robinson, Frances B. Lamb and E. J. Miller, *Ind. Eng. Chem., Anal. Ed.* 7, 265-71 (1935).

<sup>53b</sup> A. J. Hijman, *Acta Brevia Neerland. Physiol., Pharmacol., Microbiol.* 4, 148-9 (1934).

<sup>53c</sup> G. Roche Lynch, R. H. Slater and T. G. Osler, *Analyst* 59, 787-806 (1934).

<sup>54</sup> See p. 168.

should contain 0.006 to 0.012 mg. of lead. Render the sample solution distinctly alkaline with 1:1 ammonium hydroxide. If hydroxides of other metals tend to precipitate, add 0.1 gram of Rochelle salt. Dilute to about 15 cc. Add 5 cc. of 5 per cent potassium cyanide solution to avoid interference from other heavy metals.

Add 5 cc. of the green solution of the reagent and shake in a separatory funnel. Separate the carbon tetrachloride layer, which is now red, and continue to extract the sample with 1 cc. portions of fresh reagent until no further red color is obtained. Make a final extraction with 1 cc. of plain carbon tetrachloride. Combine the carbon tetrachloride extracts and extract them with 5 cc. of 1 per cent potassium cyanide solution to remove excess of the reagent. Repeat until the cyanide extract is colorless. Wash with 2 cc. of water. This leaves only the solution of the lead salt of the reagent in the carbon tetrachloride.

Dilute the carbon tetrachloride solution to 10 cc. or 20 cc. and shake with 5 cc. of 1:1 hydrochloric acid to liberate the green dithizone from the lead salt. Filter the carbon tetrachloride layer through a dry filter to remove the last trace of acid. Compare with a standard similarly prepared.

If necessary because of the small lead content of the sample, extractions and other operations can be carried out with smaller volumes of the reagents.

*Comparison as Lead Dithizone.* To the sample of about 15 cc. in a separatory funnel add the reagent in 1 cc. portions until after shaking a purple color is noticeable. This indicates the presence of excess dithizone. Add carbon tetrachloride so that with the reagent it totals 10 cc. Shake well, let separate, and draw off the carbon tetrachloride layer. Add 20 cc. of a solution prepared by diluting 10 cc. of 5 per cent sodium cyanide solution, and 5 cc. of concentrated ammonium hydroxide to 500 cc. Shake and separate the aqueous layer which contains excess of dithizone reagent. Repeat, usually twice, until the extracting solution is substantially colorless. Let the carbon tetrachloride layer stand in a stoppered tube if necessary until it is clear and compare with a series of standards similarly prepared.

#### LEAD AS THE MOLYBDATE

Lead peroxide may be converted to lead molybdate which gives an orange color with stannous chloride and a thiocyanate.<sup>55</sup> This is suitable

<sup>55</sup> S. Feinberg, *Z. anal. Chem.* **96**, 415-8 (1934).



for colorimetric estimation. As developed, it is for application to lead dioxide electrolytically deposited. The order of addition and mixing of the reagents is important. Manganese peroxide may also be present with the lead peroxide, but does not interfere. If any permanganate color develops, remove it with hydrogen peroxide.

**Sample—Lead Peroxide.** Moisten the anode having a deposit of lead dioxide, with 1:1 hydrochloric acid, drop by drop. Wash thoroughly with hot 1:100 hydrochloric acid. The precipitate goes into solution. Dilute the solution to 30–40 cc. with water and neutralize with 1:4 ammonium hydroxide. Acidify with 0.5 cc. of glacial acetic acid. Heat the solution to boiling and add 10 cc. of 0.5 per cent ammonium molybdate solution. Boil for 4–5 minutes to coagulate the precipitate. Filter on a small pad of moist filter paper laid over the opening of the stem of a filtering funnel.

Wash 5–6 times with 1:100 acetic acid. Dissolve the precipitate in boiling hot 10 per cent sulfuric acid. This will require 7–8 additions of 5 cc. portions. Cool the solution and transfer to a volumetric flask.

**Procedure—**Add 10 cc. of 5 per cent potassium thiocyanate solution and mix. Follow this with 5 cc. of 10 per cent stannous chloride in 1:4 hydrochloric acid and mix again. Dilute to 100 cc. with 10 per cent sulfuric acid. A more or less intense orange-yellow color develops which reaches the maximum in a few minutes and is stable for several hours. Compare with a standard developed at the same time which is very close in color to the sample.

**Standard—**Dissolve 0.1737 gram of molybdic oxide in 10 cc. of 1:1 sulfuric acid and dilute to 500 cc. with water. If the molybdic oxide is pure, each cc. of this solution is equivalent to 0.5 mg. of lead. It can also be standardized by gravimetric analysis for molybdenum.

#### LEAD BY HEMATIN.

Small amounts of lead in drinking water may be roughly estimated by the color reaction with hematin.<sup>57</sup> This reagent is usually considered unsatisfactory. The method is stated to detect 0.5 p.p.m. Copper, zinc and iron must be absent.

<sup>57</sup> M. R. Moffatt and H. S. Spiro, *Chem.-Ztg.* 31, 639 (1907).

**Procedure**—To 100 cc. of sample add 1 cc. of a 0.05 per cent solution of hematin in distilled water. Compare with standards similarly prepared.

#### LEAD BY SODIUM BISULFITE

In the presence of copper, nickel, iron, aluminum, silver, calcium and magnesium it is possible to estimate lead by the turbidity produced by reaction with sodium bisulfite.<sup>58-61</sup> Barium and tin must be absent. Sensitivity to 0.05 p.p.m. is claimed. A high degree of accuracy cannot be attained.

**Procedure**—To 50 cc. of sample add 10 cc. of a 20 per cent solution of sodium metabisulfite. A turbidity will form in a few minutes if 1 p.p.m. of lead is present. Comparison must be with a series of standards, or by experience with solutions of various concentrations.

#### LEAD DIOXIDE BY OXIDATION OF ANILINE

Aniline hydrochloride is converted by oxidizing agents into a purple compound. The general method for oxidizing agents as impurities in non-oxidizing materials is applicable to the determination of lead dioxide in litharge.<sup>62</sup>

**Procedure**—Prepare a stock solution containing 12 grams of colorless aniline hydrochloride, 30 cc. of concentrated hydrochloric acid and sufficient water to make 100 cc. Protect from light.

To 15 cc. of the above solution add 5 grams of the litharge sample and boil gently for 5 minutes. Cool and allow lead chloride to crystallize. Filter and compare with a series of standards prepared by adding known amounts of lead dioxide to the prepared aniline solution. The intensity of color is proportional to the lead dioxide content. Comparison by dilution or balancing has not been investigated.

<sup>58</sup> V. N. Ivanov, *Chem.-Ztg.* 38, 450 (1914).

<sup>59</sup> W. B. S. Bishop and T. Cooksley, *Med. J. Australia* 660-2 (Nov. 9, 1929).

<sup>60</sup> T. Cooksley and S. G. Walton, *Analyst* 54, 97 (1929).

<sup>61</sup> R. K. Newman, *Med. J. Australia* 781-5 (Feb. 8, 1930).

<sup>62</sup> W. V. Morgan, *J. Ind. Eng. Chem.* 11, 1055 (1919).

## CHAPTER XIX

### THALLIUM

#### THALLIUM AS THE SULFIDE

THE reactions of thallium are similar to those of lead.<sup>1</sup> It has been used with strychnine and arsenic compounds in rat poison.

**Sample**—The sample should be free from nonvolatile arsenic compounds. Ash a 2 gram sample. Extract the residue with 3 successive 10 cc. portions of hot 1:10 sulfuric acid, separating the first two times by decantation and the third time by filtration. Wash well on the filter and dilute to 100 cc.

**Procedure**—Take 10 cc. of sample solution in a 100 cc. Nessler tube and 10 cc. of standard thallium sulfate in a duplicate tube. To each add sufficient 10 per cent potassium hydroxide solution to neutralize, and 50 cc. of water. Add 3 cc. of 5 per cent sodium sulfide solution to each and dilute to 100 cc. with water. Mix well and compare by dilution or balancing. The standard and sample should not vary by more than 20 per cent.

**Standard**—Dissolve 0.1000 gram of thallium sulfate in 300 cc. of 1:10 sulfuric acid and dilute to 1 liter. The 10 cc. used as standard, subject to alteration, will therefore contain 1 mg. of thallium sulfate.

#### THALLIUM BY LIBERATION OF IODINE

A thallium salt in the presence of free chlorine or bromine is maintained in the thallic condition. So treated, after removal of excess halogen, it liberates free iodine from hydriodic acid, which is then extracted with carbon disulfide and used for estimation of thallium.<sup>2</sup> The liberation of iodine is quantitative and the extraction is efficient to 97 per cent.

Copper, lead, arsenic, iron, mercury, tungsten and molybdenum do not

<sup>1</sup> Conrad Stich, *Pharm. Ztg.* 74, 27-9 (1929).

<sup>2</sup> Paul A. Shaw, *Ind. Eng. Chem., Anal. Ed.* 5, 93-5 (1933).

interfere. Thallium chromate cannot be analysed by this method. Accuracy to 1 to 5 per cent was obtained on 0.5 mg. amounts in 20 grams of meat. Similar accuracy was obtained on thallium-coated wheat. Accuracy to 1 per cent was obtained on thallium salts. This permits of a colorimetric estimation which can be carried out much more rapidly than volumetric or gravimetric determination.

The complete determination on tissue requires 2-3 hours. On grain or samples which can be prepared by simpler methods the time required is approximately 30 minutes. For material containing 10-75 mg. of thallium per kg. a 10-20 gram sample is sufficient.

The color of iodine solutions is known to follow Beer's law. As outlined, ammonium chloride is present in the sample solution not compensated by an equal amount in the standard. This has no detectable effect on the results.

**Sample—Tissue.** Macerate a weighed sample of suitable size with 1:1 hydrochloric acid to give a fluid mass. Heat on a boiling water bath and oxidize by successive small additions of potassium chlorate until the organic matter is substantially destroyed. Let cool and filter to remove undecomposed fatty matter. Evaporate until a slight darkening occurs. Cool and transfer to a separatory funnel.

Add sufficient chlorine water to bleach the slight color and give a substantial excess. This maintains the thallium in the thallic condition. Thallous salts are insoluble in ether.<sup>3</sup> Organic matter present would reduce thallic compounds in the absence of excess oxidizing agent. The procedure isolates the thallium from many inorganic compounds and the greater part of the undestroyed organic matter. Ferric iron is extracted but later addition of phosphate removes color from that source.

Extract thoroughly with two 50 cc. portions of ether. Combine the ether extracts and evaporate in a narrow-necked flask. Add 15 cc. of water, a few drops of concentrated hydrochloric acid, and 2 cc. of concentrated sulfuric acid to the residue in the flask. Insert a short-stem funnel in the neck of the flask to prevent mechanical loss and evaporate on a hot plate to fumes of sulfur trioxide. Add concentrated nitric acid, a drop at a time, to the solution in sulfuric acid, while still on the hot plate. If necessary, fuming nitric acid may be used. Oxidation is complete when a colorless or light yellow solution is obtained.<sup>4</sup> A few drops

<sup>3</sup> A. A. Noyes, W. C. Bray and E. B. Spear, *J. Am. Chem. Soc.* 30, 515-517 (1908).

<sup>4</sup> E. L. Baldeschweiler, *Ind. Eng. Chem., Anal. Ed.* 4, 101 (1932).

of nitric acid are usually sufficient. If necessary to maintain fluidity, add a known additional volume of sulfuric acid.

Cool and add 30 cc. of 15 per cent ammonium chloride solution. Evaporate to dryness over a free flame, rotating to prevent spattering. This destroys nitrites.<sup>5</sup> If additional concentrated sulfuric acid has been added, add 15 cc. more of 15 per cent ammonium chloride for each additional cc. of acid. Dissolve in about 20 cc. of water.

*Grain containing more than 0.04 per cent thallium.* Weigh a sample of not over 0.3 gram into a narrow-neck flask. Add 2 cc. of concentrated sulfuric acid, insert a funnel in the neck of the flask and heat to sulfur trioxide fumes. Carefully add nitric acid, drop by drop, until decomposition is complete and the solution is light yellow or colorless.

Cool and add 30 cc. of 15 per cent ammonium chloride solution. Evaporate to dryness over a free flame, rotating to prevent spattering. Dissolve the residue in about 20 cc. of water.

*Grain containing less than 0.04 per cent thallium.* Treat a suitable sample as described for tissue.

*Urine containing more than 25 mg. of thallium per liter.* Measure 5 cc. or less into a narrow-neck flask. Add 2 cc. of concentrated sulfuric acid and complete as described for tissue by heating to sulfur trioxide fumes, etc.

*Urine containing less than 25 mg. of thallium per liter.* Treat a suitable sample as described for tissue.

*Thallium Salts.* While the method is essentially for materials of low thallium content, accuracy to 2 per cent in analysis of pure or nearly pure salts is obtained by dilution to approximately 0.2 mg. of thallium per cc.

**Procedure<sup>2</sup>**—Transfer the sample solution, or a suitable aliquot, to a 150–200 cc. narrow-neck flask. Add 100 cc. of concentrated hydrochloric acid and 100 grams of disodium phosphate to 900 cc. of saturated bromine-water. Add 50 cc. of this to the sample. Boil vigorously for 3 minutes while rotating over a free flame. At this stage excess bromine should have been removed. Prolonged boiling will give low results. Therefore, in routine use, the minimum time for removal of bromine should be determined. The thallic chloride present is more stable in a solution acid with hydrochloric acid. This also increases the efficiency of iodine extraction. The phosphate eliminates any effect of ferric ion.<sup>7</sup>

<sup>5</sup> G. H. Nelson, Max Levine and J. H. Buchanan, *Ind. Eng. Chem., Anal. Ed.* 4, 56 (1932).

<sup>7</sup> R. Fridli, *Deut. Z. ges. gericht. Med.* 15, 478 (1930).

Cool and transfer to a 125 cc. separatory funnel. Dilute to about 60 cc. Add 5 cc. of 0.2 per cent potassium iodide solution and 20 cc. of carbon disulfide to the separatory funnel. Stopper and shake for 15-30 seconds. Permit the layers to separate and withdraw the carbon disulfide solution of iodine. The volume of carbon disulfide used should be modified with different samples according to the probable thallium content so as to give a proper concentration of iodine after extraction. When such modification is made the volumes of bromine and potassium iodide solutions should also be modified. This need only be approximate.

Compare with a standard similarly treated, using all possible precautions to minimize evaporation of the solvent. The original work was done with a Hellige wedge-type colorimeter. In that case standards were kept for 24-48 hours in the stoppered wedge.

**Standard**—Prepare a solution of a pure thallium salt containing 0.2 gram of thallium per liter. Each cc. then contains 0.2 mg. Normally use 15 cc. of this for preparation of a standard. This will contain 3 mg. of thallium.

#### THALLIUM AS THE PHOSPHOMOLYBDATE

A thallous solution acidified with nitric acid reacts with phosphomolybdic acid to form a yellow thallous phosphomolybdate.<sup>8</sup> In the presence of excess phosphomolybdic acid this remains as a yellow hydrosol which cannot be removed by filtration or centrifuging.

A significant color is given by 0.004 mg. per cc. Lead, bismuth, mercuric and cadmium ions in large excess do not interfere under the conditions of the test. Mercurous ion gives a faint precipitate in dilute nitric acid which disappears on further addition of acid. Potassium and ammonium salts should be absent because of formation of salts with phosphomolybdic acid having low solubility. The yellow colloid dissolves in hydrochloric acid, sodium hydroxide, and sodium carbonate solutions, on warming.

Accuracy to 1-5 per cent is obtained. This is not altered by the presence of lead or bismuth, although in that case a slight increase in the amount of phosphomolybdic acid is desirable.

**Procedure**—To 5 cc. of neutral sample containing 0.002 to 0.01 mg. of thallium per cc. add 3 drops of 1:1 nitric acid and 3 drops of a 5 per cent

F. Pavelka and Hermine Morth, *Mikrochemie* 5, 30-3 (1932).

solution of phosphomolybdic acid. Mix. After 5 minutes dilute to 10 cc.

Compare with a standard developed at the same time in the same way from 5 cc. of a standard thallous nitrate solution containing 0.005 mg. per cc.

**Standard**—Dissolve 0.1304 gram of thallous nitrate in water and dilute to 1 liter. Each cc. contains 0.1 mg. of thallium. Dilute 50 cc. of this solution to 1 liter. Each cc. of this diluted solution contains 0.005 mg. of thallium.

## CHAPTER XX

### BISMUTH

#### BISMUTH AS THE IODIDE

A SOLUTION of bismuth nitrate or sulfate on addition of excess potassium iodide, assumes a yellow to orange color suitable for colorimetric comparison with a standard.<sup>1,2</sup> Bismuth is probably present as a complex iodide,  $\text{BI}_3 \cdot 3\text{KI}$ <sup>3</sup> or  $\text{BI}_3 \cdot 2\text{KI} \cdot 4\text{H}_2\text{O}$ .<sup>4</sup> The color has also been found suitable for colorimetric examination by comparison of the light absorption with a spectrophotometer.<sup>5</sup> The solution must be free from large amounts of lead, copper, tin, antimony, gold and silver.<sup>6,7</sup> Excessive amounts of nitrous, nitric and sulfuric acids must also be absent. In the presence of a small amount of iron, boiling permanently darkens the color. A large excess of sulfurous acid produces the same color as bismuth. It is removed by addition of more potassium iodide. Large amounts of alkali or ammonium salts or chlorides bleach the color. Cuprous or silver iodide may be removed by filtration without loss of bismuth. Lead iodide carries down a large proportion of the bismuth. Up to 2 mg. of lead, copper or silver will not alter the tint produced. Traces of mercury do not interfere. The presence of oxidizing agents in solution is prevented by addition of sulfurous acid, sulfite, thiosulfate or urea. Somewhat different procedures are given for organic and inorganic samples.

In a typical determination <sup>8</sup> 3 mg. of bismuth in a 200 gram sample of copper gave 2.8 mg. As small an amount as 0.01 mg. can be detected <sup>6</sup> and distinction can be made between 0.20 and 0.225 mg. Determination

<sup>1</sup> F. B. Stone, *J. Soc. Chem. Ind.* 6, 416 (1887).

<sup>2</sup> C. S. Leonard, *J. Pharmacol.* 28, 81-7 (1926).

<sup>3</sup> B. Glassman, *Z. physiol. Chem.* 172, 300-9 (1927).

<sup>4</sup> J. A. Sultzaberger, *J. Am. Pharm. Assoc.* 16, 218-21 (1927).

<sup>5</sup> H. B. Rasmussen, K. A. Jackerott and S. A. Schou, *Dansk Tids. Farm.* 1, 391-403 (1927).

<sup>6</sup> H. W. Rowell, *J. Soc. Chem. Ind.* 27, 103 (1908).

<sup>7</sup> C. Mahr, *Z. anal. Chem.* 94, 161-6 (1933).

<sup>8</sup> C. O. Jones and E. C. Frost, *Ind. Eng. Chem.* 18, 569 (1926).



in the presence of equal amounts of lead has been proposed<sup>10</sup> but is objectionable because the nature as well as intensity of color is changed.

### Inorganic Samples

**Sample—Copper, Silver and Lead Ores.** Dissolve a 10 gram sample in a mixture of 3 parts of concentrated nitric acid and 1 part of concentrated hydrochloric acid. Evaporate to dryness, bake at 200–250° and take up with 100 cc. of 1:10 hydrochloric acid. Filter to remove silica. Add potassium iodide solution to precipitate copper and a trace of silver. Add 2 cc. of concentrated sulfuric acid, evaporate to dryness, take up with 75 cc. of 1 per cent sulfuric acid and filter.

Stannous chloride has also been recommended to reduce iron in order to avoid the presence of excess sulfuric acid.<sup>11</sup>

**Arsenic, Tin or Antimony Ores.** Fuse a 10 gram sample with 50 grams of sodium carbonate and 5 grams of sulfur. Dissolve the melt in water, boil and filter. Bismuth remains as a black residue on the filter paper. Dissolve in 10 cc. of 1:1 nitric acid and dilute to about 75 cc. with distilled water.

**Copper.** Dissolve a 10 gram sample in 60 cc. of 1:1 nitric acid. Dilute with 150 cc. of water and add 10 per cent sodium carbonate solution until a slight permanent precipitate is formed. Add 1 cc. of sodium carbonate solution in excess, boil 5 minutes and let settle. All the bismuth and some copper is precipitated. Filter, wash once with distilled water and dissolve in the minimum amount of 1:1 nitric acid. Repeat the above basic salt precipitation to remove any remaining copper, dissolve the precipitate in 10 cc. of 1:1 nitric acid and dilute to about 75 cc.

**Copper containing a Trace of Bismuth.**<sup>12</sup> In the determination of traces of bismuth a special procedure is required. Dissolve a 200 gram sample of metal in nitric acid, add a crystal of ferric sulfate and make ammoniacal. Add small amounts of ammonium carbonate and sodium phosphate and boil. Allow the precipitate to settle and filter. Dissolve the precipitate in dilute sulfuric acid and pass in a stream of hydrogen sulfide. Filter. Dissolve the antimony and arsenic sulfides with potassium hydroxide or yellow ammonium sulfide solution, and again filter. Dissolve the remaining precipitate in dilute sulfuric acid, make alkaline with ammonium hydroxide and add 10 cc. of 5 per cent potassium cyanide

<sup>10</sup> H. A. B. Motherwell, *Eng. Mining J.* 104, 1091-2 (1917).

<sup>11</sup> L. C. Nickolls, *Analyst* 58, 684 (1933).

<sup>12</sup> C. O. Jones and E. C. Frost, *Ind. Eng. Chem.* 18, 597 (1926).

solution. Reprecipitate the hydrogen sulfide. The copper remains in solution. Filter. Dissolve the bismuth sulfide in 10 cc. of 1:1 nitric acid and take to fumes with 6 cc. of concentrated sulfuric acid in order to remove lead as lead sulfate. Dilute with 25 cc. of water, filter into a Nessler tube and dilute to about 75 cc.

*Copper Solutions.*<sup>13</sup> Neutralize the solution with 1:1 ammonium hydroxide. Heat to 80° and add 1:1 ammonium hydroxide until a slight permanent turbidity of basic copper salt appears. Add 1 cc. of 5 per cent solution of manganese sulfate. Mix and heat to boiling. Add 2 cc. of *N* potassium permanganate solution to the boiling liquid, drop by drop, stirring during the addition. Filter the precipitate of manganese dioxide, which will have adsorbed the bismuth. Add another portion of manganese sulfate and repeat the addition of 1 cc. of *N* potassium permanganate solution.

Dissolve the filtered precipitates in 10 cc. of 1:10 sulfuric acid by addition of hydrogen peroxide. Heat to boiling to decompose excess hydrogen peroxide and use as sample.

In a modified form<sup>14</sup> this method of separation has been used to isolate 1 part of bismuth from more than 1 million parts of copper.

*Lead and Lead Alloys.* Dissolve a 10 gram sample in 100 cc. of 1:4 nitric acid and boil to precipitate antimony and tin. Add 0.5 per cent sodium chloride solution in slight excess to precipitate silver. Add 60 cc. of boiling 1:20 sulfuric acid drop by drop with constant stirring to precipitate lead. Then add 30 cc. of 1:3 sulfuric acid. Let stand at least 1 hour to cool. Filter, washing several times by decantation with 1:20 sulfuric acid. Add 5 cc. of concentrated hydrochloric acid to the filtrate and a slight excess of ammonium hydroxide. Add 1:5 hydrochloric acid drop by drop until just acid to methyl orange, boil 1 minute and stand 1 hour. Filter bismuth. This test

ammonium hydroxide and again boiling, or by testing with potassium cyanide. Pulp the filter paper with 10 cc. of 1:3 sulfuric acid, add 30 cc. of water, boil, cool and filter from lead sulfate. Dilute to about 75 cc.

**Procedure**—Place the solution of sample or a suitable aliquot in a 100 tube. This may contain traces of antimony, arsenic, iron, lead, silver and not more than 2–3 mg. of bismuth. In a second

and Shoji Makishima, *J. Soc. Chem. Ind. Japan* 36, Suppl.

., *Anal. Ed.* 6, 189-90 (1934).

tube put the amount of nitric or sulfuric acid used in making the final solution of the sample, allowing for the aliquot taken, and dilute each to about 90 cc. To sample and standard add ten drops of a sulfurous acid solution containing 1 part of saturated solution to 2 parts of water. Add 5 cc. of 20 per cent potassium iodide solution to each. Dilute the sample to 100 cc. To the blank add sufficient standard bismuth solution to match the color of the sample and adjust the volume to 100 cc. The balancing and dilution methods may also be used. The color produced is reasonably permanent if protected from sunlight, and a series of standards may be prepared.<sup>5</sup>

**Standards**—A standard solution is prepared by dissolving 0.2321 gram of bismuth nitrate,  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ , in 50 cc. of 1:10 nitric acid and diluting to 1 liter. A similar standard may be prepared by dissolving 0.1 gram of pure bismuth or 0.115 gram of bismuth oxide,  $\text{Bi}_2\text{O}_3$ , in 5 cc. of 1:1 nitric acid and diluting to 1 liter. Sufficient acid must be added to this solution in diluting it so that the bismuth does not precipitate as the subnitrate. These standards contain 0.0001 gram or 0.1 mg. of bismuth per cc. This stock standard is used for preparing permanent standards or for duplication of the color of the sample. As a more dilute standard, 50 cc. of the stock standard are diluted to 1 liter with the addition before diluting to volume, of sufficient nitric acid to keep the solution clear. The diluted standard contains 0.005 mg. of bismuth per cc.

### Organic Samples

The iodide method for bismuth has been modified for physiological application.

**Sample—Urine.** Evaporate from 5 to 50 cc. of urine to 5 cc., add 5 cc. of concentrated nitric acid, evaporate to dryness and heat at the lowest possible temperature to oxidize but not char the organic matter.<sup>16</sup> When all of the nitric acid is driven off, remove, cool and moisten with more nitric acid. Repeat the evaporation and heating until a white residue remains. Cool, add 2 drops of concentrated nitric acid and 25 cc. of distilled water, and boil for a few minutes. If the urine contains silver salts, precipitate with a few drops of hydrochloric acid and filter. Transfer to a Nessler tube and dilute nearly to volume. The solution should be

<sup>16</sup> C. A. Hill, *Lancet* 1925, II, 1281.

clear and colorless. Add 0.3 gram of urea, dissolve and mix, and add 0.5 gram of phenazone.

A slightly different treatment for the last stage may be used. To the cold, colorless nitric acid solution add 10 cc. of a saturated solution of oxalic acid and boil until fumes appear.<sup>5</sup> This removes the last trace of nitric acid. Dilute the cooled mixture with hot water to about 20 cc. and filter. Add 1 cc. of 10 per cent sodium sulfite solution and 1 cc. of sodium citrate solution.

*Tissue or Feces.* Place 10–50 grams in a small porcelain dish, moisten with concentrated nitric acid and warm over night on a steam-heated sandbath.<sup>4</sup> Ignite over a low flame, gradually increasing to its full intensity, for 3 or 4 hours, and cool. If the residue still contains carbon, add a little nitric acid and heat again until the brown fumes are expelled. Repeat until all the carbon is oxidized. From 25 to 50 cc. of urine may be evaporated and digested in the same manner. Take up the ignited residue with concentrated hydrochloric acid and evaporate to dryness. Treat with 0.5 cc. of concentrated hydrochloric acid and 5 cc. of water. Filter, add 0.5 cc. of per cent sodium bisulfite solution, and dilute to a suitable volume.

*Tissue and Organs without Ashing.*<sup>19</sup> Reduce 30 grams of sample, such as organs, to small pieces. Dilute with water if necessary. Add 5 cc. of concentrated hydrochloric acid and 5 cc. of 1 per cent cupric chloride solution. Bring to a boil. Immerse a sheet of clean copper foil having an area not less than 4 sq. cm. for every mg. of bismuth present. Boil for 1 hour with the copper completely immersed, stirring occasionally. The bismuth is displaced and deposits on the copper.

Remove the copper foil and wash with water. Dissolve in 1:1 nitric acid, using the minimum volume for complete solution. Add 1:1 ammonium hydroxide until a faint turbidity is obtained. Add 12 drops of 1:1 hydrochloric acid and dilute with boiling water to double the volume. Heat for 1 hour on a boiling water bath to complete precipitation of bismuth oxychloride. Filter and wash the precipitate. Dissolve the precipitate from the paper with 5 cc. of 1:3 sulfuric acid. Add 0.5 cc. of approximately 5 per cent sulfur dioxide solution and dilute to a volume such that the concentration is not more than 10 mg. per 100 cc.

*Blood.*<sup>20</sup> Add 20 cc. of concentrated nitric acid to the residue in the

---

<sup>19</sup> N. A. Valyashko and P. K. Virup, *Ukrain. Khim. Zhur.* 5, Sci. Pt. 275-92 (1930).

<sup>20</sup> Eugene H. Marchling, *J. Lab. Clin. Med.* 18, 1058-61 (1933).

flask from which arsenic has been distilled.<sup>21</sup> Boil to the appearance of sulfur trioxide fumes to remove hydrazine sulfate. Cool, add 20 cc. of water and again heat to sulfur trioxide fumes. Cool. Dilute to a known volume and take a suitable aliquot for the determination. Adjust the sulfuric acid content of the standard to correspond to that of the sample.

*Bone.*<sup>4</sup> In the analysis of samples of bone, precipitate the bismuth from the hydrochloric acid solution in the usual way with hydrogen sulfide. This is to separate the bismuth from the unusually large quantity of salts present. Filter on a Gooch crucible, dissolve the precipitate in 5 cc. of concentrated hydrochloric acid, filter to remove asbestos, and dilute to volume.

Much better recoveries have been obtained from tissue and bone by ignition over a Bunsen burner than in a muffle furnace.

**Procedure**—Varying with the sample, from 10 to 50 cc. volumes may be compared with a standard. To the standard tube add the same reagents as are present in the sample and to both standard and sample add 1-5 cc. of 20 per cent potassium iodide solution. Dilute to a suitable volume, and duplicate the color of the sample by addition of one of the standard solutions previously specified.<sup>23</sup>

Hill<sup>16</sup> adds potassium iodide as small crystals in slight excess. Glassman<sup>3</sup> states that the color is orange for one part in 10,000, bright orange for one part in 40,000 and yellow for 1 p.p.m. He compares with a bichromate standard consisting of 5 cc. of 0.1 *N* solution diluted with 110 cc. of water. This color is claimed to be equivalent to 0.00333 mg. of bismuth per cc. It would presumably have that relation only to his glycerol solutions of bismuth as described in the next procedure.

*Urine. Alternative Procedure.* Another method<sup>3</sup> is to evaporate 50 cc. of urine with about 2 grams of added ammonium nitrate. Ash, and after cooling, add from 5 to 10 cc. of 20 per cent hydrochloric acid and evaporate to dryness. Again evaporate with hydrochloric acid and expel excess acid. Add 5 cc. of warm glycerol, heat on a water bath with stirring for 5 minutes, cool, and add 10 cc. of 10 per cent potassium iodide solution. Centrifuge to separate any residue insoluble in glycerol. Put 10 cc. of the clear supernatant liquid into a tube, add a definite number of drops of starch solution and 1 or 2 drops of 0.05 *N* sodium thiosulfate solution to remove the blue color, avoiding excess of the latter. Compare with similar standards in glycerol.

<sup>21</sup> See p. 226.

<sup>23</sup> See p. 215.

The use of glycerol is to avoid hydrolysis of the salt so that a large excess of acid is not required. Starch is used to detect free iodine which would cause erroneous results.

#### BISMUTH BY CINCHONINE POTASSIUM IODIDE

When cinchonine potassium iodide and a bismuth salt solution are mixed, the crimson to orange color produced is proportional in intensity to the amount of bismuth present.<sup>27</sup> The bismuth solution must be added to the reagent. It forms a complex,  $C_{19}H_{22}N_2OKIBI_3$ , which is colloiddally dispersed. If the solution is diluted too much, the color will disappear. The method is applicable to 0.03 to 0.15 mg. of bismuth.

**Sample—Ores and Alloys.** Dissolve a suitable sample in acid, according to the nature of the sample. If not already present, add 10 per cent of concentrated sulfuric acid to precipitate lead as the sulfate. Let stand, chill and filter. Wash the filter briefly, with cold 10 per cent sulfuric

alize the filtrate with 50 per cent sodium hydroxide solution and pass in hydrogen sulfide until arsenic, antimony and tin are completely precipitated, along with the bismuth. Filter the sulfides and heat to boiling with 10 per cent sodium sulfide solution to remove all arsenic, antimony and tin sulfides. Filter and wash the filter with hot 1 per cent sodium sulfide solution.

lve the bismuth and other residual sulfides from the paper with 5 cc. of hot 1:3 nitric acid. Nearly neutralize the free acidity by the addition of 1:1 ammonium hydroxide, the last portion being added drop by drop. At the end point a faint cloudiness should be visible. Add 10 cc. of 10 per cent ammonium carbonate solution with constant stirring. Digest for about 3 hours to precipitate bismuth carbonate. Decant the clear solution through a small filter. Wash the precipitate twice by decantation, using hot water containing 1 per cent of ammonium carbonate, and transfer the precipitate to the filter. Wash twice on the filter water.

Dissolve the precipitate of carbonates in 1 cc. of 1:10 nitric acid. Wash the filter with acid of the same concentration until the precipitate is completely dissolved. Use the minimum possible volume of acid for

---

<sup>27</sup> Method of W. C. Ferguson, W. W. Scott, "Standard Methods of Chemical Analysis," 4 ed., vol. I, p. 77. D. Van Nostrand Co., New York (1927).

this. Dilute the bismuth solution to a known volume and use a suitable aliquot for the procedure.

**Reagent**—Mix 10 grams of cinchonine with sufficient concentrated nitric acid to form a thick paste. Use the minimum possible amount of nitric acid. Dissolve this paste in water and dilute to about 100 cc. Dissolve 20 grams of potassium iodide separately in 500 cc. of water and add the cinchonine solution. Dilute the resulting solution to 1 liter. Let stand for 48 hours and filter.

The reagent keeps indefinitely in glass, but any suspended matter should be filtered out before use.

**Procedure**—Add 3 cc. of reagent to each of two 50 cc. Nessler tubes. Add a sufficient aliquot of the sample to one portion to give a crimson or orange hue, and dilute to a known volume. Prepare a duplicate by adding standard bismuth solution to the other tube and dilute to the same volume as the sample.

The duplication must be carried out at once; otherwise the colored compound may precipitate.

#### BISMUTH BY PHOSPHOMOLYBDOTUNGSTIC ACID

As a micro method 0.05–0.5 mg. of bismuth can be estimated in the presence of 80 mg. of cadmium or zinc by the reaction with Folin's reagent,<sup>28</sup> phosphomolybdotungstic acid.

**Procedure**—Measure a suitable volume of bismuth sample solution into a centrifuge tube. Add 2–3 drops of a fresh 4 per cent solution of pyrogallol. Heat to about 70° and add 0.33 *N* ammonium hydroxide until a distinct turbidity is visible. Heat just to boiling and add 2 drops of a 0.1 per cent solution of thymol blue in 20 per cent alcohol. Add 0.33 *N* ammonium hydroxide until an alkaline reaction is obtained with the indicator. The total volume should be about 1.5 cc. Heat 10 minutes in a boiling water bath to precipitate bismuth tannate. Let cool, dilute to about 5 cc. and centrifuge for 10 minutes at 2000–2500 r.p.m. Decant and stir up the precipitate with 5 cc. of water. Centrifuge again and decant. If necessary, repeat the washing, using 2 cc. of water. Dissolve the precipitate in 1 cc. of 1:2 hydrochloric acid and rinse into a 25

<sup>28</sup> M. Teitelbaum, *Z. anal. Chem.* 82, 366-74 (1930).

cc. flask with 15 cc. of water. Add 1 cc. of Folin's reagent<sup>29</sup> and 6 cc. of cold saturated sodium carbonate solution. Dilute to 25 cc. and compare after 30 minutes with a standard similarly treated at the same time.

**Alternative Procedure**—Treat the solution of bismuth sample in a centrifuge tube with 2 drops of 0.5 per cent sodium tartrate solution. Add 2 drops of a 0.1 per cent solution of phenolphthalein in alcohol and then 0.33 *N* ammonium hydroxide until alkaline to the indicator. Add 1 drop of 3 per cent acetic acid. Heat to about 70° and add 4 drops of 0.5 per cent oxine (hydroxyquinoline) acetate solution. Heat just to boiling and add 0.3 cc. of saturated sodium acetate solution. Heat on a water bath for 10 minutes and centrifuge. From that point continue as by the first procedure.

#### BISMUTH BY THIOUREA

When thiourea is added to solutions containing bismuth, a yellow color is produced by the formation of complex bismuth-thiourea compounds such as  $\text{Bi}(\text{CSN}_2\text{H}_4)_5(\text{NO}_3)_2\text{OH}$ ,  $\text{Bi}(\text{CSN}_2\text{H}_4)_2\text{Cl}_3$  and  $\text{Bi}(\text{CSN}_2\text{H}_4)_3\text{Cl}_3$ . This qualitative reaction has been developed into a quantitative method.<sup>30</sup> Sufficient nitric acid must be present to prevent hydrolysis of the bismuth salt without having much effect on the color produced.

In high concentrations silver, mercury, lead, copper, cadmium and tin give white precipitates. In more dilute solutions they give neither color nor precipitate. Copper salts are decolorized by the reagent. Antimony gives a color similar to that from bismuth. This interference can be removed by the addition of hydrofluoric acid, which forms a complex with the antimony. Interference by ferric ion is prevented by reduction to ferrous ion. High concentrations of chromium, nickel and cobalt give colored ions. In their presence the bismuth is separated by reduction, by precipitation with hydrogen sulfide, or by adsorption on a suitable carrier.

The method is most satisfactory at a concentration of 0.2–4.0 mg. of bismuth per 100 cc. The accuracy is then to 1 to 4 per cent. As small an amount as 0.01 per cent of bismuth can be detected.

**Procedure**—Evaporate the sample solution containing bismuth until acid has been substantially all removed. Take up the moist residue with 10 cc. of 1:15 nitric acid. If ferric iron is present, add 0.1 gram of

<sup>29</sup> See p. 183.

<sup>30</sup> C. Mahr, *Z. anal. Chem.* **94**, 161-6 (1933).



hydrazine sulfate and bring to a boil to reduce the iron. Add solid thiourea until an excess is present. This precipitates foreign metals. About 1 gram will normally be required. Filter on an inorganic filter and wash the filter with 1:15 nitric acid saturated with thiourea. Dilute the filtrate to 25 cc. with 1:15 nitric acid saturated with thiourea and compare with a standard similarly prepared. The comparison is best made in sunlight or with a blue filter over the lamp.

The color develops at once and is stable for several hours. Sulfur turbidity gradually develops, and in the presence of much lead, copper, or arsenic, may appear within an hour. The method has also been applied with color glasses as standards.<sup>31</sup>

#### BISMUTH AS IODOBISMUTHATE OF QUININE

The iodobismuthate of quinine in acetone solution has a yellow to orange-yellow color.<sup>32</sup> The method has been reported as unreliable.<sup>33</sup> It is therefore given only very briefly.

**Reagent**—The iodoquinine reagent contains 1 gram of quinine sulfate, 3-4 drops of sulfuric acid and 2 grams of potassium iodide in 100 cc. of aqueous solution.<sup>34</sup>

**Procedure**—To 10 cc. of a solution of bismuth in 10 per cent nitric acid add 2 cc. of the iodoquinine reagent and 8 cc. of acetone. Compare with a standard solution treated similarly.

As a modification, add 3 cc. of a 10 per cent solution of gum arabic to 5 cc. of sample, then 2 cc. of iodoquinine reagent.<sup>35</sup> Cuny and Poirot obtained accuracy to 2 to 3 per cent.

#### BISMUTH BY *o*-HYDROXYQUINOLINE

A reddish-orange insoluble complex is formed between bismuth and *o*-hydroxyquinoline. Under suitable conditions it may be used for colorimetric estimation.<sup>36</sup> The method is a modification of that with quinine.<sup>32</sup>

<sup>31</sup> C. Mahr, *Z. anal. Chem.* 97, 96-9 (1934).

<sup>32</sup> C. E. Laporte, *J. pharm. chim.* 28, 304 (1923).

<sup>33</sup> H. B. Rasmussen, K. A. Jackerott and S. A. Schou, *Dansk Tids. Farm.* 1, 291-403 (1927).

<sup>34</sup> P. Aubrey, *J. pharm. chim.* 25, 15 (1922).

<sup>35</sup> L. Cuny and G. Poirot, *J. pharm. chim.* 28, 215 (1923).

<sup>36</sup> R. Sazerac and J. Pouzergues, *Compt. rend. soc. biol.* 109, 79-82, 370-1 (1932).

As in that method, precipitation can be prevented by addition of acetone, or the color extracted with acetone and amyl acetate. Cyclohexanol is also a suitable extraction medium.

Solvent extraction is not necessary by the procedure that follows if 0.25 mg. of bismuth is present. The method will detect 0.000005 mg.

**Procedure**—Mix equal volumes of a 4 per cent solution of potassium iodide, and a 2 per cent solution of *o*-hydroxyquinoline in mixed 0.1 per cent nitric and 0.1 per cent sulfuric acid.

To 5 cc. of sample solution add 0.5 cc. of the above reagent, and 4 cc. of a mixture of 2 volumes of acetone and 1 volume of amyl acetate. Shake and let stand. Compare the color of the solvent layer with that of a similar layer produced from a standard bismuth solution.

#### BISMUTH BY POTASSIUM THIOCYANATE

A yellow color appears in an acid solution of bismuth on addition of potassium thiocyanate.<sup>38</sup> Heinrichs and Hertrich report an error in the colorimetric determination of iron in minium due to the presence of bismuth. Bismuth may be removed and determined colorimetrically before determining iron.

**Procedure**—Precipitate bismuth from solution by means of hydrogen sulfide.<sup>39</sup> Dissolve the bismuth sulfide by warming with a small volume of 1:1 nitric acid. Precipitate with ammonium hydroxide, filter and redissolve the bismuth oxide in a small volume of 1:5 sulfuric acid. Place in a Nessler tube and dilute to 50 cc. with a 20 per cent solution of potassium thiocyanate. Into a second Nessler tube introduce the same amount of 1:5 sulfuric acid, dilute to nearly 50 cc. with 20 per cent potassium thiocyanate solution and add a standard solution of bismuth until the colors match. Adjust the volumes of each to 50 cc.

#### BISMUTH AS THE SULFIDE

When a solution of a bismuth salt of the proper acidity is treated with hydrogen sulfide in the presence of a colloid such as gelatine the yellow color of the sulfide can be used for colorimetric estimation.<sup>39a</sup> In

<sup>38</sup> H. Heinrichs and M. Hertrich, *Chimie & industrie* 14, 696 (1925); *Glastech. Ber.* 2, 112 (1924).

<sup>39</sup> See next method.

<sup>39a</sup> F. Malengreau and G. Delrue, *Arch. internat. méd. exptl.* 35-46

the experimental development with pure bismuth salts the color was found to be proportional to the amount of bismuth present. The method is suitable for amounts of 0.002–0.1 mg. per cc. in the final sample. For 0.2 to 0.8 mg. in the sample used the accuracy is within 5 per cent.

**Sample—Organic.** Wet-ash a suitable sample with nitric and sulfuric

Evaporate as much of the acid as possible and dilute to about cent acidity. Filter to separate calcium sulfate and any other insoluble material. To the filtrate add a few cc. of saturated ammonium chloride solution. Add 1:3 ammonium hydroxide until neutral to methyl orange, to precipitate bismuth oxychloride. Let stand for several hours. Withdraw a portion of the supernatant liquid and test qualitatively to be sure that precipitation is complete. Filter and wash the precipitate with water. Dissolve the precipitate from the paper in 5 cc. of 1:9 hydrochloric acid. Add 1 cc. of 1 per cent gelatine solution and dilute with water to a suitable volume.

**Procedure**—Pass hydrogen sulfide into the solution of sample until the color is fully developed. Compare with a selected volume of standard diluted to 5 cc. with 1:9 hydrochloric acid and then to the same volume as the sample with water, and similarly treated.

*In alkaline solution.*<sup>39b</sup> To the sample solution add 1 gram of sodium potassium tartrate and 10 cc. of 1 per cent gum arabic solution. Dilute to about 75 cc. and add concentrated ammonium hydroxide drop by drop until a definite excess is present and a drop on a spot plate is alkaline to phenolphthalein. Add 0.5 cc. of 10 per cent sodium sulfide solution and mix. Dilute to 100 cc. Compare with a known volume of standard similarly treated.

**Standard**—Dissolve 0.1 gram of pure bismuth or 0.115 gram of bismuth oxide,  $\text{Bi}_2\text{O}_3$ , in 50 cc. of 1:1 hydrochloric acid and dilute with water to 500 cc. This contains 0.2 mg. of bismuth per cc. as the chloride in 1:9 hydrochloric acid.

#### BISMUTH NEPHELOMETRICALLY BY SODIUM STANNITE

For bismuth in dilute solution addition of sodium stannite gives a dispersion suitable for nephelometric estimation.<sup>39c</sup>

<sup>39b</sup> Takmaro Yamamoto, *Bull. Inst. Phys. Chem. Research (Tokyo)* 13, 1265-6 (1934).

<sup>39c</sup> Luigi Malossi, *Rend. accad. sci. (Napoli)* 2, 83-90 (1932).

**Sample—Copper.** Dissolve 20 grams of copper in concentrated nitric acid. Evaporate on a water bath with occasional additions of concentrated hydrochloric acid until no further brown fumes appear. Dilute as necessary and filter. Add a saturated solution of ammonium carbonate to the filtrate until it is neutral to methyl orange. Filter and wash the precipitate which contains bismuth, lead, iron and small amounts of copper. Dissolve the precipitate in hot 1 : 9 hydrochloric acid using as small a volume as possible. Concentrate or dilute to 10 cc.

**Procedure—**Add 5 cc. of hot, filtered 0.5 per cent agar agar solution to 10 cc. of sample. Prepare a sodium stannite solution by adding 20 per cent sodium hydroxide to 20 per cent stannous chloride solution until the precipitate just redissolves. Compare the resulting turbidity with that produced from known amounts of bismuth standard <sup>39d</sup> diluted to 10 cc. with 1 : 9 hydrochloric acid.

---

<sup>39d</sup> See p. 215.

## CHAPTER XXI

### ARSENIC

#### ARSENIC BY THE GUTZEIT METHOD

IN THE determination of arsenic by the Gutzeit method the arsenic is reduced to arsenious acid and then, by treatment in a hydrogen generator, to arsine. The arsine is led over paper treated with mercuric chloride or better yet with mercuric bromide and the stain produced compared with stains produced by standard amounts of arsenic.<sup>1</sup>

Numerous modifications<sup>2</sup> have been proposed. A survey shows that many of these cannot be used by the average analyst with accuracy until he attains considerable experience in their application.<sup>3</sup>

Wet-ashing has been reported as unsatisfactory on plant materials unless followed by distillation of the arsenic as the chloride.<sup>4</sup> Reduced sensitiveness in the presence of mercury, bismuth, copper, iron and selenium has been reported.<sup>5</sup> This is avoided by redistillation as the chloride.<sup>6</sup>

The presence of pyridine derivatives in the sample causes results<sup>7</sup> to be 5 to 50 per cent low. This has been traced to failure of tin to adhere to the zinc used for evolution, slow evolution of hydrogen, and incomplete conversion of the arsenic to arsine. A method of separation of the arsenic to avoid such interference has been devised and is given as applied to tobacco.

When wet digestion methods are used, low temperature or oxidizing conditions must be maintained.<sup>8</sup> When charring of the sample takes place, arsenic may be reduced from the pentavalent to the trivalent form.

<sup>1</sup> "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists," 2nd ed., p. 171 (1925).

<sup>2</sup> H. Schröder and W. Lühr, *Z. Untersuch. Lebensm.* 65, 168-76 (1933).

<sup>3</sup> W. F. Clarke, *J. Assoc. Official Agr. Chem.* 11, 438-42 (1928).

<sup>4</sup> R. B. Deemer and J. A. Schricker, *J. Assoc. Official Agr. Chem.* 16, 226-32 (1933).

<sup>5</sup> J. F. Reith, *Pharm. Weekblad* 70, 369-73 (1933).

<sup>6</sup> J. F. Reith, *Pharm. Weekblad* 69, 1358-74 (1932).

<sup>7</sup> C. R. Gross, *Ind. Eng. Chem., Anal. Ed.* 5, 58-60 (1933).

<sup>8</sup> Roe E. Remington, E. Jack Coulson and Harry von Kolnitz, *Ind. Eng. Chem., Anal. Ed.* 6, 280-1 (1934).

The vapor pressure of arsenious oxide<sup>9</sup> is substantially zero below 250°, 32 mm. at 275° and 144 mm. at 338°, which is the boiling point of sulfuric acid. If heated to vigorous fuming while reducing material is present, substantial loss may occur. This is avoided by keeping the temperature low until oxidation is complete.

The presence of difficultly oxidizable substances is taken care of by dry-ashing. Pyridine derivatives, such as those from tobacco, give satisfactory results by that method.

Various workers have recommended the use of a paper disc.<sup>10-14</sup> Several devices have been developed for clamping the sensitized paper in a standard position over the end of the exit tube, usually as a disc.<sup>15-24</sup> Because of their complexity and since they have not yet been adopted as standard, they will not be described here. Standards keep for six months if the bromide is used but will fade after a week or ten days if the chloride is used. If the stain produced has been contaminated with antimony it will be longer and lighter in color. This may be confirmed by exposing the stain to the fumes of hydrochloric acid. A stain from antimony will fade on exposure while an arsenic stain will be intensified.<sup>25</sup> It has been recommended that instead of zinc in the generator, arsenic-free iron be used for the production of hydrogen.<sup>26</sup> This will produce the desired arsine but not the undesirable stibine or phosphine. The use of a small amount of stannous chloride in the generator serves to sensitize the zinc and to absorb any iodine liberated. More than a trace of fluorine must be eliminated from the sample.

Stick zinc has been reported to give more uniform evolution of hydro-

<sup>9</sup> A. F. Heck, *Soil Sci.* 28, 225 (1929).

<sup>10</sup> Bird, *Analyst* 26, 181 (1901); *Chemist and Druggist* 1901, 600.

<sup>11</sup> T. F. Harvey, *Chemist and Druggist* 1905, 168.

<sup>12</sup> Hill and Collins, *Chemist and Druggist* 1905, 548.

<sup>13</sup> "British Pharmacopœia," Appendix VI, pp. 501-20 (1914).

<sup>14</sup> C. E. Lachale, *Ind. Eng. Chem., Anal. Ed.* 6, 256-8 (1934).

<sup>15</sup> J. R. Stubbs, *Analyst* 52, 700-1 (1927).

<sup>16</sup> John White, *Ibid.* 52, 701-2 (1927).

<sup>17</sup> C. H. Cribb, *Ibid.* 52, 701 (1927).

<sup>18</sup> For discussion of earlier references see H. Droop Richmond, *Ibid.* 53, 90 (1928).

<sup>19</sup> A. S. Dodd, *Ibid.* 53, 152 (1928).

<sup>20</sup> A. D. Comrie and T. J. Ward, *J. Inst. Brewing*, 34, 530-3 (1928).

<sup>21</sup> C. H. Manley, *Analyst* 54, 30 (1929).

<sup>22</sup> A. J. Lindsey, *Ibid.* 55, 503-4 (1930).

<sup>23</sup> T. J. Ward, *Ibid.* 55, 630 (1930).

<sup>24</sup> G. H. Davis, *Ibid.* 56, 30 (1931).

<sup>25</sup> W. S. Allen and R. M. Palmer, *Disc. 8th Inter. Cong. App. Sci.*, 27, 4

<sup>26</sup> Antipas Ericsson, *Svensk Farm. Tids.* 17, 468, 491 (1913).

gen than granular zinc.<sup>27,28</sup> The same accuracy is obtainable with stick, 20-mesh and 30-mesh zinc with all other conditions the same.<sup>28a</sup> The method is more accurate than the titrametric bromate method.<sup>29,27</sup> Granular aluminum is also suitable for operation of the generator.<sup>30</sup> With hydrochloric acid alone its rate of evolution of hydrogen is uneven. Stannous chloride renders it uniform. The amount of stannous chloride required is greater than when zinc is used. The lead acetate on cotton or glass wool absorbs any hydrogen sulfide evolved.

It is essential that the temperature of the evolution and absorption apparatus be the same in preparation of sample and standards.<sup>31</sup> This is to maintain the moisture content of the absorbents comparable. A lower temperature in the generator will produce shorter and more intense colors. Lower temperatures in the absorption apparatus produce faint, long stains which are less definite.

Unless there is a reduction period of 30 minutes at 25°, reduction of amounts equivalent to more than 0.02–0.03 mg. of arsenious oxide may be incomplete.<sup>28</sup> This is a higher temperature than the present standard procedure provides.

**Sample—General.**<sup>24</sup> Weigh a suitable amount of well-mixed sample, such as 5 grams of dry substance, 25 grams of pulped vegetables, or 50 grams of liquid into a casserole. Add 10–15 cc. of concentrated nitric acid. Cover and heat until vigorous action ceases. Cool and add 10 cc. of concentrated sulfuric acid. Heat until the mixture turns dark, add 5 cc. of nitric acid and heat further. Continue the addition of 5 cc. portions of nitric acid and heating until the liquid is colorless or yellow when taken to sulfur trioxide fumes. Evaporate to 5 cc., cool, dilute with 10–15 cc. of water and again evaporate to fumes of sulfuric trioxide. Cool, dilute with water and when cool dilute to a definite volume, 25, 50 or 100 cc.

**Inorganic.** If large amounts of inorganic material are present from which the arsenic must be separated, a distillation as the chloride with absorption in dilute alkali is advisable.<sup>34-36</sup> For this, place the inorganic

<sup>27</sup> W. Catesby Jones, *J. Assoc. Official Agr. Chem.* 16, 325-9 (1933).

<sup>28</sup> C. R. Gross, *J. Assoc. Official Agr. Chem.* 16, 398-403 (1933).

<sup>28a</sup> C. R. Gross, *J. Assoc. Official Agr. Chem.* 18, 189-91 (1935).

<sup>29</sup> W. C. Taber, *J. Assoc. Official Agr. Chem.* 14, 436-7 (1931).

<sup>30</sup> Louis P. Mayrand, *J. Am. Pharm. Assoc.* 20, 637-43 (1931).

<sup>31</sup> H. Heidenhain, *J. Assoc. Offic. Agr. Chem.* 11, 107-12 (1928).

<sup>34</sup> I. Bang, *Biochem. Z.* 161, 193 (1925).

<sup>35</sup> H. Kleinmann and F. Pangritz, *Ibid.* 185, 44-62 (1927).

<sup>36</sup> T. Callan and S. G. Clifford, *Analyst* 55, 102-9 (1930).

sample or sample of ash containing 0.02 to 5 mg. of arsenic in a 500 cc. distilling flask with a low side arm. Close the flask with a rubber stopper previously soaked in hot 1:3 hydrochloric acid. Connect to a condenser with a rubber stopper similarly treated. Connect an exit tube from the condenser to a wash bottle to deliver the distillate in 25 cc. of approximately 0.1 *N* sodium hydroxide solution. Gas from this wash bottle should pass through a second 25 cc. portion of 0.1 *N* sodium hydroxide solution.

To the sample add 2 grams of ferrous sulfate, 2 grams of potassium chloride and 0.2 gram of potassium bromide. Add 50 cc. of concentrated sulfuric acid and distil. The distillation should be so carried out that the solution does not boil but hydrogen chloride is evolved and carries over the arsenic chloride with it. The temperature should rise to 90° in about 10 minutes when distillation will be complete.<sup>36a</sup> Sulfur dioxide fumes do not interfere.

If the residue from the distillation is to be used for lead determination, replace the ferrous sulfate with hydrazine sulfate.

*Organic.*<sup>35</sup> If the material is moist or is in solution, make alkaline and evaporate to dryness on a water bath. When dry, rub to a fine powder. To ash put 20 grams of dry powder into a Kjeldahl flask and treat with concentrated nitric acid. Add to the nitric solution an equal volume of concentrated sulfuric acid and a few crystals of copper sulfate. Add fuming nitric acid from time to time if necessary. Nitrous fumes escape slowly and no carbonizing occurs. The process may take from 7 to 8 hours. Hastening will cause loss of arsenic. Remove the remainder of the nitric acid and decompose nitrosyl sulfuric acids by several evaporations with water.

If necessary to separate arsenic from the ash, add 20 grams of ferrous sulfate, 20 grams of potassium chloride and 2 grams of potassium bromide. Distil in the apparatus by the method specified for inorganic samples but using 100 cc. of 0.1 *N* sodium hydroxide solution in each receiver. Evaporate this distillate to about 50 cc. and neutralize with sulfuric acid for use as sample.

*Carius Oxidation*<sup>38</sup> of *Organic Samples*. As an alternative method of destruction of organic samples they are heated in a sealed tube in a bomb furnace by the usual Carius method. One cc. of fuming nitric acid will oxidize 3 cc. of blood serum, 100 cc. of spinal fluid or 0.5 g. of dry tissue. After heating at 260° for 2 hours, remove and evaporate the

<sup>36a</sup> W. Catesby Jones, *J. Assoc. Official Agr. Chem.* 17, 202-4 (1934).

<sup>38</sup> M. Vinograd, *J. Am. Chem. Soc.* 36, 1548-51 (1914).



contents of the tube with sulfuric acid to expel nitric acid. Cool and dilute with water. The amount of sulfuric acid used for expulsion of nitric acid must be taken into account in making up the test solution in the Sanger-Black-Gutzeit apparatus.

*Bomb Decomposition of Organic Samples.* Bomb decomposition is probably the most rapid and efficient method for decomposition of organic samples. A special micro bomb for the purpose has been designed<sup>38a</sup> and applied to samples for arsenic.<sup>38b</sup> The equipment is shown in Figure 70 with dimensions.

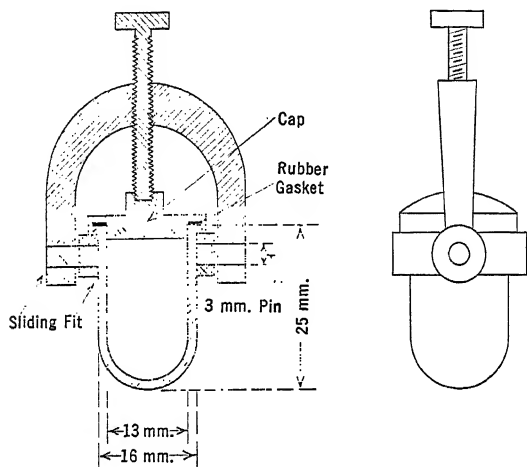


FIG. 70

Micro Bomb for Decomposition of Organic Samples

For use place 20–25 mg. of sucrose and the dry sample containing 1.5–3.0 mg. of arsenic in the bomb constructed of nickel. Add about 1 gram of sodium peroxide and close the bomb. Mix the charge by shaking the bomb violently for 2 minutes. Tap the bomb on the table to make sure that the charge is all in the bottom of the cup. Ignite the charge by heating with the tip of a small, hot flame for 35–40 seconds. Let stand in the air for 5 seconds to cool and immerse in cold distilled water.

Remove the lid from the bomb and rinse thoroughly into a test tube. Transfer the cup to the tube and heat to dissolve the fusion. Remove the cup and rinse with hot water. Evaporate the solution in the tube

<sup>38a</sup> Fred E. Beamish, *Ind. Eng. Chem., Anal. Ed.* 5, 348-9 (1933).

<sup>38b</sup> Fred E. Beamish and H. L. Collins, *Ind. Eng. Chem., Anal. Ed.* 6, 379-80 (1934).

to about 10 cc., adding beads to prevent bumping. This insures destruction of peroxides. Neutralize the solution with 1:1 hydrochloric acid. Dilute to a known volume and use an aliquot as sample for the determination.

Combustion of samples containing arsenic can also be carried out in an oxygen bomb designed for the purpose,<sup>38c</sup> but precipitation of suspended particles is essential to complete recovery.

*Fruit Peelings.*<sup>39,40</sup> Heat the sample gently with 15–20 cc. of concentrated sulfuric acid and 35 cc. of concentrated nitric acid. After the reaction has quieted heat more strongly, adding more nitric acid, until the solution is light yellow but not brown. Avoid charring. Boil gently for 10 minutes, cool, add 100 cc. of water and evaporate to sulfur trioxide fumes. Heat for 10 minutes, cool and dilute to a standard volume. Use an aliquot.

*Gelatine.* Weigh 10 g. of gelatin into a beaker. Add 50 cc. of 1:3 hydrochloric acid and heat on a water bath until dissolved. Add 20 cc. of saturated bromine water, let digest for an hour, remove and cool.<sup>41</sup> Dilute to 100 cc., mix and filter. Use an aliquot for the procedure.

*Phosphoric Acid.*<sup>42</sup> Weigh 5 grams of acid into the generating flask. Modify the procedure by omission of potassium iodide.

*Metallic Copper.*<sup>43</sup> Dissolve in nitric acid, precipitate arsenic as ferric arsenate, dissolve the precipitate in dilute sulfuric acid and use for the procedure. The amount of acid used for solution of the ferric arsenate must be considered in making up standard strips.

*Tissue or Other Biological Material.* By the treatment indicated under "Mercury as Colloidal Sulfide"<sup>43a</sup> arsenic, antimony and mercury are obtained as a precipitate of mixed sulfides. A part or all of this precipitate may be used for determining arsenic.

After washing, dissolve the arsenic and antimony from the precipitate on the filter with 10 cc. of yellow ammonium sulfide solution, and evaporate to dryness on the water bath. Dissolve the residue in a few cc. of 1:1 nitric acid, evaporate to dryness and add 3 cc. of concentrated sul-

<sup>38c</sup> F. P. Carey, G. Blodgett and H. S. Satterlee, *Ind. Eng. Chem., Anal. Ed.* 6, 327-30 (1934).

<sup>39</sup> H. Heidenhain, *J. Assoc. Official Agr. Chem.* 11, 107-12 (1928).

<sup>40</sup> J. W. Barnes and C. W. Murray, *Ind. Eng. Chem., Anal. Ed.* 2, 29-31 (1930).

<sup>41</sup> R. M. Mehurin, *Ind. Eng. Chem.* 15, 942 (1923).

<sup>42</sup> William H. Ross, C. B. Durgin and R. M. Jones, *J. Ind. Eng. Chem.* 14, 533-5 (1922).

<sup>43</sup> See p. 213.

<sup>43a</sup> See p. 178.

furic acid. Heat on a sand bath until sulfur trioxide fumes appear, cool and dilute to a convenient volume.

*Blood.*<sup>44</sup> By this method both bismuth and arsenic are obtained in a form suitable for use. Mix 5 cc. of blood in a Kjeldahl flask with 27 cc. of concentrated nitric acid and 3 cc. of concentrated sulfuric acid. When oxidation is substantially complete, add 30 cc. of concentrated nitric acid to complete the digestion. When the solution is colorless, cool and add 70 cc. of water. Boil to destroy traces of nitrosyl sulfuric acid and cool.

Connect to a condenser through a safety bulb and add 10 grams of sodium chloride, 1 gram of sodium bromide and 1 gram of hydrazine sulfate. The latter is for reduction of pentavalent arsenic. Distil the arsenic trichloride in the usual way.<sup>45</sup> Pass a very slow stream of air to facilitate distillation and start the heating slowly. Use 10 cc. of concentrated nitric acid and 5 cc. of bromine water in the receiver. When distillation is complete, evaporate this to dryness. Add 6 cc. of 1:1 sulfuric acid and heat on a sand bath to sulfur trioxide fumes. Cool and add 5 cc. of water. Again heat to sulfur trioxide fumes. Cool and dilute with water for use as sample.

*Tobacco.*<sup>46</sup> Heat 200 grams of tobacco with fuming nitric acid until organic matter is completely decomposed and all chlorides expelled. Evaporate repeatedly with sulfuric acid until all nitric acid is removed. Cool, dilute with water and when cold dilute to a standard volume. Transfer an aliquot <sup>7</sup> of the diluted digested solution representing about 0.2 mg. of arsenic trioxide to a 400 cc. beaker and dilute to about 200 cc. Add concentrated ammonium hydroxide until distinctly alkaline. Add 20 cc. of a phosphoric acid solution prepared by dilution of 19 cc. of the 85 per cent grade to 1 liter. Slowly add 25 cc. of a magnesia mixture containing 55 grams of hydrated magnesium chloride, 55 grams of ammonium chloride and 88 cc. of concentrated ammonium hydroxide per liter. Stir during this addition. Add 5 cc. of concentrated ammonium hydroxide, stir and let stand for at least 15 minutes. Filter through an 11 cm. paper. Wash the precipitate on the paper four times with 15 cc. portions of 1:9 ammonium hydroxide. Finally wash with 10 cc. of water. Drain for 15 minutes with occasional shaking to promote complete filtration.

Dissolve the precipitate in 40 cc. of 1:4 hydrochloric acid, adding

<sup>44</sup> Eugene H. Maechling, *J. Lab. Clin. Med.* 18, 1058-61 (1933).

<sup>45</sup> See p. 227.

<sup>46</sup> H. Popp, *Z. angew. Chem.* 41, 838-9 (1928).

the acid in small portions. Collect the solution in a 100 cc. volumetric flask. Wash the filter with 50 cc. of water and dilute to volume. Use 5 to 20 cc. aliquots for the determination, making allowance for the acid already present.

This method of separation of the arsenic from interfering pyridine derivatives in tobacco is also applicable to many other types of samples where the presence of pyridine derivatives is suspected.

*Bones.*<sup>48</sup> All the arsenic can be extracted from bone powder by leaching with dilute sodium hydroxide solution.

*Sprayed Foliage.*<sup>49</sup> Incomplete digestion with 10 per cent nitric acid or 20 per cent hydrochloric acid for 30 minutes, filtering and washing gave practically the same results as complete digestion with nitric and sulfuric acids.

*Malt.*<sup>20</sup> The most accurate results are obtained by ashing as for organic samples and dissolving the ash. Approximate values can be obtained directly. Pale and amber malts yield 71 to 83 per cent of the arsenic present. For this method use 10–25 grams of malt as sample and multiply the arsenic determined by 1.3.

*Hops, Grain and Sugar.*<sup>20</sup> Place 12 grams of sample, 150 cc. of water and 5 cc. of 20 per cent magnesium nitrate solution in a casserole. Boil gently, evaporate to dryness and ignite carefully until free from carbon. When cool, dissolve soluble material in 20 cc. of 10 per cent hydrochloric acid and 1 drop of 20 per cent stannous chloride solution. Heat on a water bath 5 minutes, cool, dilute to 50 cc. and filter. Use aliquots of the filtrate for the determination.

*Food Dyes.*<sup>52</sup> For amaranth, naphthol, yellow S, tartrazine, guinea green B and light green SF yellowish, use the following method: Dissolve 10 grams of the dye in 250 cc. of water and add 10 cc. of saturated bromine-water. Make the solution alkaline with 1–2 cc. of concentrated ammonium hydroxide. Add 20 cc. of a solution containing 100 grams of crystallized trisodium phosphate and 50 grams of phosphoric acid per liter. Add magnesia mixture containing 55 grams of hydrated magnesium chloride, 55 grams of ammonium chloride and 88 cc. of concentrated ammonium hydroxide, until the phosphate is fully precipitated, then 5 cc. in excess. Make this addition slowly, stirring meanwhile, on the basis of a previous determination of the quantity required to react

<sup>48</sup> G. Popp, *Z. angew. Chem.* 41, 856-8 (1928).

<sup>49</sup> J. M. Ginsburg, *J. Econ. Entomol.* 21, 588-92 (1928).

<sup>52</sup> "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists," 2nd ed., pp. 158-9 (1925).

with the phosphate. Add 10 cc. of concentrated ammonium hydroxide and let stand for at least 30 minutes. Filter through an 18 cm. filter paper and wash the paper with 1:10 ammonium hydroxide until it is free of dye. Wash with about 5 cc. of water. Let the paper and precipitate drain for about 30 minutes.

Remove the filtrate and dissolve the precipitate with 40 cc. of 1:4 hydrochloric acid. To do this, add the acid in small portions and use the generator bottle as receiver. Use this solution as sample. It already contains the necessary amount of acid.

For erythrosine dissolve 18 grams of the dye in 425 cc. of water. Add 5 cc. of saturated bromine-water and 20 cc. of 1:4 hydrochloric acid. Mix, filter and treat 250 cc. of the filtrate, corresponding to 10 grams of dye-stuff, with concentrated ammonium hydroxide until slightly alkaline. This will usually require about 5 cc. Proceed as for the other dyestuffs starting with the addition of phosphate solution.

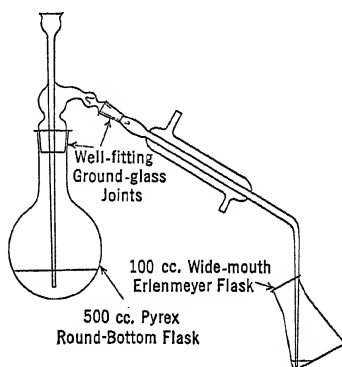


FIG. 71

Apparatus for Distillation of Arsenic

*Separation of Selenium and Arsenic from Soils by Distillation.*<sup>53</sup> A distillation method has been developed<sup>54</sup> which separates arsenic, selenium and germanium from all other elements by distillation with hydrobromic acid. By separation of the selenium according to known procedures a sample suitable for estimation of arsenic is obtained. The method is applicable to samples other than soil by suitable modification.

Transfer 50 grams of air-dried soil to the distillation flask of the apparatus shown in Figure 71. Add 10 cc. of a solution of 10 cc. of bromine in 100 cc. of concentrated hydrobromic acid, a few cc. at a time, with shaking. This avoids loss by frothing if carbonates are present. Continue addition of this solution until it is in excess, the amount depending on the amount of organic matter in the soil. Add concentrated hydrobromic acid to bring the volume of this reagent up to

<sup>53</sup> W. O. Robinson, H. C. Dudley, K. T. Williams and Horace G. Byers, *Ind. Chem., Anal. Ed.* 6, 274-6 (1934).

<sup>54</sup> A. A. Noyes and W. C. Bray, "Qualitative Analysis for the Rare Elements," Macmillan Co., New York (1927).

75–100 cc. The greater volume is used if much carbonate is present. The hydrobromic acid must be of a grade which will be completely decolorized by sulfur dioxide.

Connect the flask with the condenser. Place 2–3 cc. of saturated bromine water in the receiver with the adapter dipping below its surface. The first few cc. of distillate should also carry over a couple of cc. of bromine. If that amount does not distil over, add more to the flask. Excess bromine is harmful only in producing an excess of sulfuric acid in a later operation.

Heat gently at first and finally with full heat until 30–50 cc. of distillate have collected. Add 50 cc. more of the bromine in hydrobromic acid and repeat the distillation. A third distillation may be necessary, unless experience shows that all of the arsenic (and/or selenium) is in the first distillate.

Pass sulfur dioxide into the distillate until the color of the bromine has been destroyed. Add about 0.5 gram of hydroxylamine hydrochloride. Stopper the flask loosely and heat on a steam bath for an hour. Let stand over night at room temperature to precipitate the selenium as a red or black precipitate.

Filter the selenium on an inorganic filter and wash with concentrated hydrobromic acid containing a trace of hydroxylamine hydrochloride. The residue on the filter is to be used for the estimation of selenium.

The arsenic and germanium are present quantitatively in the filtrate from the selenium. Add 10 cc. of concentrated nitric acid and evaporate to about 25 cc. Cool, add 5 cc. of concentrated sulfuric acid and concentrate to sulfur trioxide fumes. When cool, dilute with water and use as sample. The required amount of acid is already present.

*Pyrites and Other Sulfides.*<sup>53</sup> Grind the sample to pass a 100-mesh screen. Heat 100 grams of concentrated nitric acid in a 300 cc. dish on the steam bath. Weigh out a 10 gram sample. Add this in small portions to the hot acid. Stir thoroughly between additions as long as brown fumes are evolved. When half the sample has been added, increase the nitric acid by 25 cc. and continue. After the reaction is complete add 15 cc. of concentrated sulfuric acid and evaporate all the nitric acid.

Add 5 drops of 30 per cent hydrogen peroxide and stir vigorously. When the hydrogen peroxide has decomposed add 75 cc. of a mixture of 100 cc. of 48 per cent hydrobromic acid with 10 cc. of bromine and transfer to a distillation flask. Complete as for soil.<sup>56</sup>

<sup>53</sup> See p. 233.

*Water.*<sup>53</sup> Make a 1-10 liter sample alkaline with sodium peroxide. Evaporate to dryness. Take up the residue with 100 cc. of 48 per cent hydrobromic acid to which 1-5 cc. of bromine has been added, depending on the amount of organic matter in the water. Transfer to a distillation flask and complete as for soil.<sup>58</sup>

*Vegetable Matter.*<sup>53</sup> Stir 100 grams of well ground and mixed sample into a concentrated solution of 25 grams of magnesium nitrate. Add 5 grams of magnesium oxide and dry the mass on the water bath. Complete in an oven at 105°. Ignite slowly in a muffle to a uniform grey ash. Triturate the ash with 100 cc. of 48 per cent hydrobromic acid and 2 cc. of bromine. Transfer to a distilling flask and complete as for soil.<sup>58</sup>

*Tissue.*<sup>53</sup> Cut the material into small pieces and drop into cold 30 per cent hydrogen peroxide. When frothing ceases, warm on a water bath. Add concentrated nitric acid in small amounts until decomposition is nearly complete. Then add magnesium nitrate equal to 5 per cent of the weight of the sample. Evaporate to dryness and ash at a low temperature. Take up the ash with a suitable amount of 48 per cent hydrobromic acid containing 2 per cent by volume of bromine. Transfer to a distilling flask and complete as for soil.<sup>58</sup>

*Combustible Materials by Dry Ashing.*<sup>8</sup> A detailed technique for combustion with an enclosed torch and washing of the combustion gases with suitable absorbing liquids has been developed.<sup>64</sup> This is described in its application to estimation of iodine.<sup>65</sup>

Set up the apparatus and handle it with modifications as below. Substitute 2 cc. of 1:1 nitric acid in each absorption bottle in place of the sodium hydroxide solution. Add a third wash bottle, as the absorption of arsenic trioxide is less efficient than that of iodine. After combustion, transfer the water and ash of the cup to a beaker. Rinse these with 5 cc. of 1:100 nitric acid. Also add the contents of the absorption bottles to the beakers and rinse out the bottles with water.

Concentrate to a small volume to insure oxidation and to expel chlorine. This is of particular importance with sea foods. Add 5-20 cc. of concentrated sulfuric acid and cover the beaker with a watch glass. Evaporate to sulfur trioxide fumes. From a properly conducted combustion there should be no darkening with sulfuric acid. If any does

<sup>53</sup> See p. 233.

<sup>64</sup> Harry von Kolnitz and Roe E. Remington, *Ind. Eng. Chem., Anal. Ed.* 5, 33 (1933).

<sup>65</sup> See pp. 553-8.

occur, add a few drops  
and heat until this has  
to a known volume and use the sample or an  
which follows.

*Oils.*<sup>8</sup> Absorb a suitable sample in arsenic-free cotton and proceed as for combustible materials by dry ashing.

**Apparatus**<sup>67,68</sup>—A 50–100 cc. wide-mouthed bottle or flask is used as generator. Fit this by a rubber stopper to a glass tube 1 cm. in diameter and 6 cm. long. In this tube place a roll of heavy filter paper  $4.5 \times 16$  cm. soaked in 20 per cent lead acetate solution and dried. To this tube connect by a rubber stopper a similar tube filled with cotton wrung out of 20 per cent lead acetate solution. This should be uniformly moist in the center and saturated with 10 per cent lead acetate solution for the cotton.<sup>69</sup> Sea sand or fine sand may also be used.<sup>28a</sup> It minimizes the formation of shorter, deeper-colored, sharper bands.

with 20 per cent lead acetate solution. The tube should be fitted with a rubber stopper long to contain the strip of paper. The tube should be soft and free from coating materials.

**Test Paper**—To prepare this, dry a heavy close-textured, unsized paper 100° and keep in a desiccator until needed.<sup>70</sup> This is to insure uniform coloring. Just before using cut<sup>71</sup> into strips exactly 1 cm. wide. Saturate with a 5 per cent solution of lead acetate in alcohol. Drain and dry in a desiccator until the strips are dry. Do not use at high temperatures. Produces poor and dim colors.

<sup>67</sup> Sanger-Black-Gutzeit apparatus. R. C. Griffin, "Technical Methods of Analysis," p. 53. McGraw Hill Book Co., New York (1927).

<sup>68</sup> Chas. R. Sanger and Otis F. Black, *J. Soc. Chem. Ind.* 26, 1115-23 (1907).

<sup>69</sup> T. J. Ward, *Analyst* 51, 457 (1926).

<sup>70</sup> G. Kemmerer and H. H. Schrenk, *Ind. Eng. Chem.* 18, 707 (1926).

<sup>71</sup> For a suitable cutter for large numbers see E. I. Green, *Ind. Eng. Chem.* 19, 424 (1927).



**Procedure**—In the 3 mm. tube place one of the prepared strips of test paper. After running a blank on the reagents, introduce an aliquot of the solution containing approximately 0.03 mg. of arsenious oxide into the wide-mouthed bottle of the apparatus.

To the sample solution in the generating bottle add 20 cc. of 1:4 sulfuric acid or 1:3 hydrochloric acid if that amount of acid is not already present. Add water if necessary to make a volume of about 40 cc. Add 4 cc. of 20 per cent potassium iodide solution and heat to about 90°. Add 3 drops of a 40 per cent solution of stannous chloride in concentrated hydrochloric acid and heat for 10 minutes.<sup>72</sup> Cool to about 5° in ice water, add about 15 grams of stick or 20-mesh arsenic-free zinc and connect the apparatus as described so that the gas evolved will be filtered through cotton or glass wool before passing over the sensitized mercuric bromide paper. Keep the generator in ice water for fifteen minutes, then remove and let run for an hour longer.

A more recent procedure<sup>31</sup> is to cool to 30°, add the zinc, then suspend the entire apparatus in a water bath at 30° for 18 hours.

Compare the stain with stains produced in the same manner by known amounts of arsenic, using portions of a standard solution containing 0.001, 0.002, 0.005, 0.010, 0.025, and 0.030 mg. of arsenious oxide, adding water and acid so as to have the same volume and concentration of acid as in the final unknown solution. A blank test should not show more than 0.001 mg. of arsenious oxide.

Instead of direct comparison with standards, it has been recommended<sup>74</sup> that the length of the stains by a given procedure be plotted on graph paper and values interpolated. The values for 5, 10, 15 and 20 mm. lengths are sufficient to interpolate the intermediate values and will give results accurate within 5 per cent of those obtained by direct comparison. Standard stains may be

Mercuric Bromide  
Paper

Lead Acetate  
Cotton



Lead Acetate  
Paper

Solution

Stick Zinc

FIG. 72

Sanger-Black-  
Gutzeit Apparatus  
for Arsenic  
Determination

<sup>72</sup> Omission of this heating has been recommended. H. Heidenhain, *J. Assoc. Official Agr. Chem.* 11, 107-12 (1928).

<sup>74</sup> Bertram D. Thomas, *Ind. Eng. Chem.* 26, 356 (1934).

dipped in melted paraffin and mounted or recorded photographically.<sup>74a</sup>

To determine very slight traces of arsenic remove the paper stained by the action of arsine, and immerse in a 10 per cent potassium iodide solution.<sup>75</sup> A very faint stain turns orange-brown thus becoming more easily observable. Wash and dry the paper before comparing with a standard scale prepared by the same method of treatment. By examination in ultraviolet light instead of daylight the sensitivity is strikingly increased.<sup>76</sup>

**Special Sample and Procedure for Coal**<sup>74a</sup>—Decomposition of coal or coke with nitric acid followed by fuming sulfuric acid has been used<sup>76b</sup> but entails some loss. Therefore a modified Gutzeit method is required.

Mix 1 gram of coal sample passing 100-mesh with 0.8 gram of a mixture of 5 parts of sodium carbonate, 3 parts of magnesium oxide and 1 part of potassium nitrate. Place the mixture in a low porcelain or platinum crucible and set in a cold muffle furnace. Raise the temperature to 700–750° in 1 hour and heat until the carbon is all burned out. This should require a total of 2–3 hours. By keeping the temperature under 750° there is little trouble with sintering. Remove the crucible from the muffle. Use 2–3 cc. of water from a wash bottle to wet the cold residue and wash down the sides of the crucible. Add concentrated sulfuric acid drop by drop with stirring until the mixture is just acid to litmus.

Transfer the contents of the crucible to the bottle of a Gutzeit apparatus. Add 3.5 cc. of concentrated sulfuric acid. Add 2 cc. of a solution of 84 grams of ferric ammonium sulfate in 100 cc. of 1:4 sulfuric acid containing 10 grams of sodium chloride. Add 0.5 cc. of 40 per cent stannous chloride in concentrated hydrochloric acid. Dilute to approximately 45 cc. with distilled water and stir until thoroughly mixed. Place the bottle in a water bath at 25°. Add stick zinc to the bottle, connect with the usual apparatus and let run for 50–60 minutes.

**Standard**—Dissolve 1 gram of arsenious oxide,  $\text{As}_2\text{O}_3$ , in 25 cc. of 20 per cent sodium hydroxide solution, add about 500 cc. of air-free water, acidify with dilute sulfuric acid and dilute to 1 liter with air-free water. One cc. of this solution contains 1 mg. of arsenious oxide. At the time of use prepare a more dilute solution containing 0.001 mg. per cc. For this purpose dilute 20 cc. of the stock solution to 1 liter, remove 50 cc. and dilute this further to 1 liter.

<sup>74a</sup> E. S. Hertzog, *Ind. Eng. Chem., Anal. Ed.* 7, 163-5 (1935).

<sup>75</sup> J. Cribier, *J. pharm. chim.* 24, 241 (1921).

<sup>76</sup> Alfred A. King, *J. Soc. Chem. Ind.* 47, 304 (1928).

<sup>76b</sup> L. Archbutt and P. B. Jackson, *J. Soc. Chem. Ind.* 20, 448-50 (1901).

## ELECTROLYTIC GUTZEIT METHOD

Modifications of the preceding method have been developed in which hydrogen is generated by the electrolysis of sulfuric acid.<sup>77</sup> This eliminates the use of zinc which is difficult to obtain sufficiently free from arsenic for the accuracy necessary in some determinations. The method is claimed to be sensitive to 0.0002 mg. of arsenious acid.

**Apparatus**—The apparatus consists of a U-tube of Pyrex glass, one arm serving as cathode chamber and the other as anode chamber. That used by Fink is 11 cm. in height, has an inside diameter of 1 cm., and a capacity of 15 cc. It is fitted with hollow ground-glass stoppers in which platinum wires are sealed for connections to the electrodes. The cathode is a strip of pure sheet lead  $2.5 \times 6.25$  cm., and the anode a strip of platinum foil of the same dimensions. Both electrodes are rolled to fit the inner diameter of the apparatus and are held in place by copper clips soldered to copper wires, which in turn are welded to the platinum wires sealed in the glass stoppers.

Fused to the outlet from the cathode side is a tube 5 mm. in diameter and 4 cm. long. It communicates with the interior by means of an opening in the glass stopper. This side tube is connected to a 4 mm. glass tube about 24 cm. long, the lower portion of which contains the glass wool and the upper part the mercuric bromide paper. Fused to the anode chamber is a tube 4 cm. long which serves for the escape of oxygen. This apparatus is suspended in a water bath which serves to reduce the rise in temperature caused by the generation of arsine. The cell is connected in series with a set of resistance lamps, the latter connected in parallel. As many cell units as desired may be placed in series to make possible several determinations at one time. If leaves or fruit are to be analysed it is necessary to use an apparatus with a capacity of 50 to 75 cc. with correspondingly larger electrodes.

After each determination the electrodes are sensitized by immersing for several minutes in warm dilute nitric acid, then washing thoroughly with water. The apparatus is cleaned similarly, with the aid of a test tube brush, and rinsed several times with water.

**Procedure**—To determine arsenic in organic matter such as muscle tissue or the organs of insects, grind the material with 2–3 cc. of 12.5 per cent sulfuric acid and dilute with this acid to 10 cc. Place directly

<sup>77</sup> D. E. Fink, *J. Biol. Chem.* 72, 737 (1927).

in the apparatus without further treatment. Arsenic from other sources is concentrated in 1-2 cc. of solution, added to 10 cc. of 12.5 per cent sulfuric acid and introduced into the apparatus for electrolysis. Add 1 or 2 cc. of amyl alcohol if the sample is organic, to prevent foaming. Immerse in a water bath at 20°. Electrolyze with a direct current of 1 ampere and 11 volts. If the solution contains arsenious acid or sodium

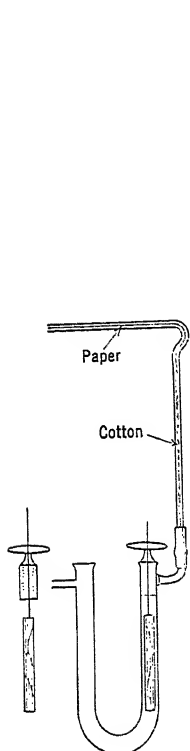


FIG. 73

Electrolytic  
Gutzeit Apparatus

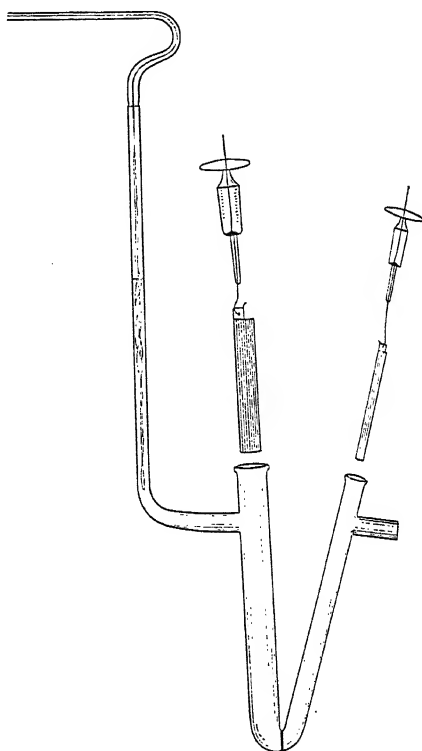


FIG. 74

Modified Electrolytic Gutzeit Apparatus

arsenite to the extent of 0.01 mg. or less, electrolysis for half an hour is usually sufficient. For higher concentrations, or if arsenic is in the form of arsenic acid or sodium arsenate, the time required is considerably greater.

**Standard**—If the sample consists of insect tissue, grind similar tissue with known amounts of arsenic and treat the same way. Other-

wise add known amounts of a standard solution of arsenic to 10 cc. of 12.5 per cent sulfuric acid and electrolyze as above.

#### MODIFIED ELECTROLYTIC GUTZEIT METHOD

A later development of the electrolytic method is that of Osterberg.<sup>79</sup> To eliminate the necessity for the diffusion of the arsenious acid from the anode to the cathode chamber, a diaphragm of alundum is sealed in to separate the two chambers. The reduction is more complete and more rapid, since the arsenious acid is always on the reducing side of the apparatus.

**Apparatus**—The apparatus is as shown in Figure 74. The two arms of the V-shaped tube serve respectively as cathode and anode chambers. In the bend between them is sealed an alundum disc. The arms are 16 cm. in length; the cathode chamber has an internal diameter of 25 mm. and the anode chamber 10 mm. The cathode consists of a rounded lead sheet, 4 by 9 cm.; the anode is a platinum foil 26 by 70 mm. The electrodes are fastened to the glass stoppers by means of sealed-in platinum wires, which make connection through mercury to copper wires, cemented through the tops of the stoppers and connected with the source of the current. The stopper of the cathode chamber only, has to be ground. The cathode chamber holds approximately 8 cc. In other respects the apparatus is very similar to the one previously described.

#### ARSENIC BY THE COCAINE-MOLYBDATE OR STRYCHNINE-MOLYBDATE REAGENT

This is a nephelometric method dependent on the reaction of pentavalent arsenic with a high molecular-weight material to produce a colloidal dispersion. Phosphates must be absent. A cocaine-molybdate reagent<sup>80</sup> may be used. The use of an equivalent strychnine-molybdate reagent, well known for phosphorus determination,<sup>81</sup> has also been recommended.<sup>82</sup>

This determination is applicable to aliquots of solution containing from 0.0005 to 0.06 mg. of arsenic. The error amounts to 2 to 3 per cent in quantities of 0.005 mg.

<sup>79</sup> A. E. Osterberg, *J. Biol. Chem.* **76**, 19 (1928).

<sup>80</sup> H. Kleinmann and F. Pangritz, *Biochem. Z.* **185**, 14 (1927).

<sup>81</sup> P. A. Kober and G. Egerer, *J. Am. Chem. Soc.* **37**, 2373-81 (1915).

<sup>82</sup> Luigi Belladen, Ugo Scazzola and Renato Scazzola, *Ann. chim. applicata* **23**, 517-21 (1933).

**Sample**—This is the chloride absorbed in alkali.<sup>83</sup> Oxidize the arsenic trichloride to pentachloride in alkaline solution with a few drops of 30 per cent hydrogen peroxide. Warm and make faintly acid to phenolphthalein with hydrochloric acid. Evaporate to a small volume and filter through asbestos. Dilute to a standard volume.

**Procedure**—To a 20 cc. aliquot of the above solution and to a suitable standard add 5 cc. of the fresh reagent. After standing a short time compare in the nephelometer with a standard similarly prepared.

**Reagents**—To prepare the cocaine-molybdate reagent, mix 1 part by volume of 1 per cent potassium molybdate solution, 2 parts by volume of 0.1 *N* hydrochloric acid, and 1 part by volume of a 2 per cent cocaine solution.

To prepare the strychnine-molybdate reagent, dissolve 6 grams of sodium carbonate in 140 cc. of water. Gradually add 19 grams of molybdic oxide and heat until dissolved. Add 50 cc. of concentrated hydrochloric acid and 20 cc. of 2 per cent solution of strychnine sulfate. It is stable for several months.

#### ARSENIC BY QUININE ARSENOMOLYBDATE

This nephelometric method, similar to the preceding, gives a colloidal sol.<sup>84</sup>

**Sample**—To a 50 cc. sample free from metals precipitated by hydrogen sulfide from acid solution, add 10 cc. of concentrated sulfuric acid. The arsenic present should be between 0.008 and 0.035 mg. Saturate with hydrogen sulfide and let stand for 24 hours. Filter through asbestos and wash. Dissolve the precipitate in a mixture of 10 cc. of concentrated nitric acid and 10 cc. of saturated bromine water. Evaporate to dryness on a water bath.

**Reagent**—(1) Dissolve 3.5 grams of sodium carbonate in 50 cc. of water in a 100 cc. volumetric flask. Add 9.5 grams of molybdic oxide and heat on a water bath until the oxide dissolves. Let cool and dilute to 100 cc. (2) Treat 1.3207 grams of arsenious oxide with 5 cc. of concentrated nitric acid and evaporate nearly to dryness on a water

<sup>83</sup> See p. 227.

<sup>84</sup> D. Chouchak, *Ann. chim. anal. chim. appl.* [2] 4, 138 (1922); *Analyst* 47, 317 (1922).

bath. Dissolve in water and dilute to 1 liter. Dilute 10 cc. of this solution to 1 liter.

Dissolve 0.5 gram of neutral quinine-hydrochloride in 10 cc. of distilled water. Add 5 cc. of the dilute arsenic solution (2) prepared as above. Add 10 cc. of 1:3 nitric acid and then 1 cc. of the sodium molybdate solution (1), stirring continuously during the addition of the latter. A precipitate first forms, then redissolves on further addition of molybdate. The final solution is a slightly cloudy, opalescent mixture. Dilute to 120 cc., mix and filter through a filter paper first washed with nitric acid and then with water.

By the method of preparation the reagent is saturated with quinine arsenomolybdate and therefore is extremely sensitive. It will keep several months without change.

**Procedure**—Take up the residue in 20 cc. of the reagent. At the same time add 0.5 cc. of 1:3 nitric acid and 2.5 cc. of an arsenic solution containing 0.01 mg. of arsenic per cc. to 2 cc. of water and 20 cc. of reagent. Dilute standard and sample to 25 cc. and compare at the end of 15 minutes.

#### ARSENIC BY SODIUM HYPOPHOSPHITE

This nephelometric method depends on the reduction of arsenic pentachloride with sodium hypophosphite in strongly acid solution,<sup>85</sup> Bougault's reagent.

This reagent reduces organic substances such as sugars, gums, starch and dextrins when conditions are favorable for reduction to glucose.<sup>86</sup> A brown coloring formed in the presence of these organic materials is probably humic acid. The method is therefore applicable only in the absence of organic matter.

**Sample**—Prepare sample as described elsewhere<sup>87</sup> so that the final concentration of arsenic is at least 0.0005 mg. per cc.

**Reagent**—To prepare Bougault's reagent dissolve 20 grams of sodium hypophosphite in 20 cc. of distilled water, add 200 cc. of concentrated hydrochloric acid and filter through cotton to separate sodium chloride

<sup>85</sup> M. Delaville and J. Belin, *Bull. soc. chim. biol.* 9, 91 (1927).

<sup>86</sup> R. Guyot, *Bull. soc. pharm. Bordeaux* 53, 337-40 (1914).

<sup>87</sup> See pp. 227-36.

crystals. Leave in a cool place until a second deposition of sodium chloride occurs, and filter. The reagent must be clear.

**Procedure**—To a 5 cc. sample and to 5 cc. of a suitable standard solution add 5 cc. of the reagent. Put the two tubes into a boiling water bath for 30 minutes, cool and compare in a nephelometer.

#### ARSENIC BY FORMATION OF MOLYBDENUM BLUE

Arsenic reacts with ammonium molybdate to form the arsenomolybdate which may be reduced to form a blue complex of molybdenum. The reaction is the same as that with phosphorus,<sup>88</sup> both arsenomolybdate and phosphomolybdate being reduced to the compound called "molybdenum blue." It has been recommended that arsenic be determined by comparison with phosphate standards.<sup>89</sup> Stannous chloride has been stated to be the most satisfactory reducing agent although others may be used.<sup>90</sup> The best development of color is obtained with 4 cc. of 2.5 per cent ammonium molybdate and 4 cc. of 10 *N* sulfuric acid per 100 cc.<sup>91</sup> One variation<sup>92,92a</sup> calls for reduction of the molybdate with molybdenum powder to avoid the necessity of having excess reducing agent present. The resulting color on reaction with arsenic is unchanged for 7 to 10 days.

This is probably the most sensitive and accurate method known for arsenic. It has not been sufficiently investigated to be adopted as a standard method in place of the Gutzeit method. Silica does not interfere up to 700 p.p.m. If the acidity is high a low intensity of color is obtained due to incomplete formation of arsenomolybdate. Increase of ammonium molybdate requires a corresponding increase of sulfuric acid. The proper concentration of acid is very important. Up to 6 p.p.m. ferric ion does not seriously interfere. The tendency is to decrease the total color and produce greenish tints. If the iron exceeds 6 p.p.m. a special procedure is necessary. Titanium up to 20 p.p.m. does not interfere. Larger amounts do. Its effect is to delay development of color, which may be compensated by allowing time for the color to develop. Aluminum and

<sup>88</sup> See p. 486 et seq.

<sup>89</sup> W. R. G. Atkins and E. G. Wilson, *Biochem. J.* 20, 1225 (1926).

<sup>90</sup> Cf. Hydrazine sulfate, E. H. Maechling and F. B. Flinn, *J. Lab. Clin. Med.* 15, 779-82 (1930).

<sup>91</sup> Emil Truog and A. H. Meyer, *Ind. Eng. Chem., Anal. Ed.* 1, 136-9 (1929).

<sup>92</sup> S. R. Zinzadze, *Z. Pflanzenernähr. Düngung Bodenk.* 16A, 129-84 (1930); *Udobrenie i Urozhai* 3, 827-32 (1931).

<sup>92a</sup> Ch. Zinzadze, *Ind. Eng. Chem., Anal. Ed.* 7, 230 (1935).



manganese do not interfere in considerable amounts. Nitrate has no effect up to 100 p.p.m.; at 200 p.p.m. it decreased the color 10 per cent. The use of nitric acid in place of sulfuric acid gave about 10 per cent less color, which faded rapidly. Up to 1000 p.p.m. calcium and magnesium do not interfere.

If necessary, the distillation of arsenic as the chloride<sup>93</sup> may be used for separation from other elements, after which satisfactory results are obtained.<sup>4</sup> The method is applicable for quantities of 0.002 to 10 mg. For biological and toxicological work precipitation as the sulfide has also been recommended from solutions obtained by wet ashing.<sup>95</sup>

A blank determination is essential because of the wide distribution of arsenic and phosphorus in reagents and equipment used. The corresponding arsenotungstate is not reduced under these conditions.

**Sample—Phosphorus absent.** Dissolve by procedures already given.<sup>96</sup> Dilute to 50 cc. and clarify by suitable means if turbid or colored. Otherwise neutralize any excess of acidity or alkalinity in 50 cc. and proceed.

**Phosphorus present.** Proceed with one 50 cc. sample exactly as though phosphorus were absent. To another add an excess of concentrated hydrochloric acid and reduce arsenic by passing hydrogen sulfide through the solution. As an alternative, add an acid solution of sodium sulfide. Boil off the excess of hydrogen sulfide and add paper pulp. Shake vigorously for ten minutes and filter. This removes any precipitated sulfur. Neutralize the resulting solutions and proceed. Obtain the value of each in terms of arsenic and by difference the amount of arsenic in the sample.

**High iron content.** If more than 6 p.p.m. of iron are present acidify a 100 cc. sample with 8 cc. of 1:2.6 sulfuric acid, heat to about 80° and reduce in a Jones reductor filled with cadmium. After reduction is complete, dilute the solution and washings to 150 cc. Titrate a portion for free acid. To 75 cc. add sufficient sulfuric acid to increase the acidity to that of 4 cc. of 10 *N* sulfuric acid, in other words to a value such that when diluted to 100 cc. the solution will be 0.4 *N*.

**Samples ignited with magnesium nitrate.** If organic matter in the sample has been destroyed by this method, 4 cc. of 1:2.6 sulfuric acid may be used in dissolving the residue. In that case omit the addition of this acid in the procedure for development of color.

<sup>93</sup> See p. 227.

<sup>95</sup> Guy E. Youngburg and Jason E. Farber, *J. Lab. Clin. Med.* 17, 363-6 (1932).

<sup>96</sup> See pp. 227-36.

**Procedure—By Stannous Chloride Reagent.** Dilute 5 cc. of standard solution containing 0.005 mg. of arsenic per cc. to about 90 cc. Dilute the neutral sample to about 90 cc. To each add 4 cc. of 1:2.6 sulfuric acid and 4 cc. of 2.5 per cent ammonium molybdate solution, and mix well. Add 6 drops of 2.5 per cent stannous chloride solution in 1:9 hydrochloric acid and dilute each to 100 cc. Mix well and compare by dilution or balancing. The color develops at once. The standard contains 0.00025 mg. of arsenic per cc. If the colors differ too greatly prepare another standard using 2 cc. of the 0.005 mg. per cc. arsenic solution. This standard will contain 0.0001 mg. of arsenic per cc. If allowed to stand 10 minutes, add 1 drop more of stannous chloride to each before comparing. This may be repeated up to 1 hour.

**By Reduced Molybdenum Reagent.** Transfer 0.5–30 cc. of the standard and unknown to 50 cc. volumetric flasks. They should contain 0.005–0.3 mg. of arsenic. Add 5 drops of saturated  $\alpha$ -dinitrophenol solution and neutralize with 2 per cent sodium bicarbonate solution or with *N* sulfuric acid to a faint yellow color. Add 5 cc. of the reagent,<sup>97a</sup> dilute to 40 cc. and mix. Heat in a boiling water bath for 30 minutes and cool. Dilute to 50 cc., mix and compare.

**Standard—**Dissolve 0.0200 gram of arsenic pentoxide,  $\text{As}_2\text{O}_5$ , in 5 cc. of 2 per cent sodium bicarbonate solution and dilute to about 100 cc. Add 6 cc. of *N* sulfuric acid and 3 drops of 0.1 *N* potassium permanganate solution. Dilute to 1 liter. This stock solution contains 0.02 mg. of arsenic pentoxide per cc. Dilute 25 cc. of it to 1 liter to give a standard containing 0.005 mg. per cc., or to 50 cc. to give one containing 0.01 mg. per cc.

#### ARSENIC BY STANNOUS CHLORIDE

Arsenic produces a reddish brown color in solution with stannous chloride.<sup>98</sup> The color develops more promptly in the presence of 0.00001 *M* mercuric chloride<sup>99</sup> and more than that amount will cause turbidity. The sensitivity as a qualitative test is greater than that of the Gutzeit or Marsh tests. The method, which is only semi-quantitative, has been adapted to determination of the arsenic in *ferrum reductum* and may be susceptible of complete quantitative adaptation.

<sup>97a</sup> See pp. 506-7.

<sup>98</sup> E. S. Peack, *Pharm. J.* 13, 130 (1901).

V. Bernard King and F. E. Brown, *Ind. Eng. Chem., Anal. Ed.* 5, 168-71 (1933).

## ARSENIC BY SILVER NITRATE

In this modification<sup>100</sup> of the Gutzeit method arsenic is reduced to arsine and passed through the tubes with lead acetate paper. The gas is then passed through a tube containing a few crystals of silver nitrate. The black stain produced on these crystals is to be compared with stains produced by known amounts of arsenic in a similar way. The standards may be sealed in the tubes in which they were produced and kept indefinitely. Vapors<sup>102</sup> from the generator of a Gutzeit apparatus are also led through a 0.5 per cent solution of lead acetate in 5 per cent sodium hydroxide to remove hydrogen sulfide and then through a 0.01 *N* solution of silver nitrate. The coloration produced is compared with standards.

For increased sensitivity the vapors containing a trace of arsenic are led through a narrow tube and impinge on silver nitrate paper.<sup>103</sup> The action is thus confined to a small area. The form of the method described<sup>103a</sup> as illustrative is one best adapted as a rapid control method of moderate rather than extreme accuracy. In it the arsine reduces the silver nitrate to a red-brown colloidal silver solution. The method is suited to estimation of quantities of the order of 0.01 mg. Samples and standards must be prepared and compared in diffused light.

In a modified form<sup>103b</sup> the absorption is by aqueous silver nitrate and the arsenic absorbed is oxidized to arsenate and determined by the molybdenum blue reaction.

**Sample—Wine.** Transfer 20 cc. of sample to a porcelain dish and neutralize with sodium carbonate. Slowly stir in 0.2 gram of calcined magnesium oxide and 0.8 gram of magnesium nitrate. Evaporate to dryness on a water bath and dry further at 110°. Put the dish in a cold muffle and slowly heat to a dull red in 2 hours. Continue to heat to a bright red and heat until ashing is complete. Dissolve the ash in 15 cc. of 1:9 sulfuric acid and use as sample. Loss in ashing may be as high as 25 per cent of the arsenic present. Allowance for this must be made in calculating the result. It is a major source of error in the method.

**Apparatus.** A typical design of apparatus is shown in Figure 75. The evolution is carried out in a 2 by 9 cm. test tube, A. The absorption

L. Moreau and E. Vinet, *Compt. rend.* 158, 869-71 (1914).

<sup>102</sup> A. F. Judd, *J. Am. Pharm. Assoc.* 2, 961-2 (1913).

<sup>103</sup> F. Martin and J. Pien, *Bull. soc. chim.* 47, 646-54 (1930).

<sup>103a</sup> M. Schluty, *Chimie & Industrie*, Special No. 244-6 (April, 1934).

<sup>103b</sup> Gert Taubmann, *Arch. exptl. Path. Pharmacol.* 176, 751-6 (1934).

tubes, D, are made from glass tubing of about 12 mm. internal diameter. Draw out 2 mm. glass tubing to a fine capillary for conducting the gas from the evolution tube A through tube C into the absorption tube D. Blow a bulb B, on the evolution end after fitting it through the stopper, and provide orifices on the sides for the passage of gas. For use fill this bulb B with cotton impregnated with saturated lead acetate solution to absorb hydrogen sulfide.

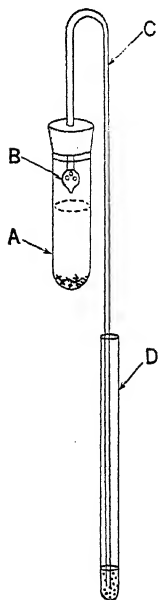


FIG. 75

Apparatus for  
Absorption of  
Arsine in Silver  
Nitrate Solution

**Procedure**—Transfer the sample to the tube used for evolution. Into a similar tube introduce 10 cc. of the 1:9 sulfuric acid, 1.5 cc. of standard solution containing 0.01 mg. of arsenic per cc. and 3.5 cc. of water. To each add 1 gram of arsenic-free zinc, and 1 drop of 0.25 per cent nickel sulfate solution as catalyst. Connect with the apparatus at once. The absorption tubes contain 2 cc. of pure glycerine and 2 drops of 20 per cent silver nitrate solution. Let the apparatus run for at least 1 hour to complete the reaction.

**Standard**—Dissolve 0.416 gram of sodium arsenate,  $\text{Na}_2\text{AsO}_4 \cdot 7\text{H}_2\text{O}$ , in water and dilute to 1 liter. This contains 0.01 mg. of arsenious oxide per cc.

#### ARSENIC AS THE TRISULFIDE

Arsenic is distilled from acid solution as arsenic trichloride and deposited on a piece of cloth wet with hydrogen sulfide solution, as arsenic trisulfide.<sup>104</sup> The appearance of the layer of yellow trisulfide is compared with deposits similarly produced by known amounts of arsenic.

Differences of 0.1 mg. are ordinarily recognizable. By varying the field for deposition, the amount of arsenic which may be determined by this method can be varied. Unless the fabric is quite porous due to use of a relatively coarse cloth or to needle perforations, not over 0.5 mg. of arsenic can be deposited before the cloth is clogged.

**Sample**—Preparation of the sample has been given in detail under the Gutzeit method.<sup>105</sup>

<sup>104</sup> J. Mai, *Z. anal. Chem.* 41, 362 (1902).

<sup>105</sup> See pp. 227-36.

**Procedure**—Place about 1 cc. of sample containing 1 mg. or less of arsenic in a 25 mm. test tube with 10 cc. of concentrated hydrochloric acid. Fit this with a 2-holed stopper containing inlet and outlet tubes. The inlet tube should dip below the surface of the liquid. The outlet tube should be above the surface and be formed with a double bend so as to connect to an inverted Gooch funnel by means of rubber tubing. Make the outlet tube tip slightly toward the test tube so that only vapors will pass through to the funnel. Coat the rim of the funnel with a thin layer of vaseline to prevent creeping. Fasten over the funnel by means of a rubber band a piece of bleached cotton cloth in which a few holes have been punched with a needle.

Pass a stream of carbon dioxide at 2-4 bubbles per second into the solution containing arsenic. After 5 minutes adjust a crystallizing dish of freshly saturated hydrogen sulfide solution so that the surface just wets the cloth stretched over the inverted funnel. Warm the arsenic solution so that the acid boils slightly, adding suitable materials to prevent bumping. The arsenic trichloride passes over with gaseous hydrogen chloride and forms arsenic trisulfide quantitatively on meeting the inner surface of the cloth wet with hydrogen sulfide solution. Do not heat more than an hour. Remove the dish from beneath the cloth, detach the funnel, carefully remove the stained cloth and let dry in the air. Compare with standard stains.

**Standards**—Prepare standards by a similar procedure on cloth of the same area. As standard solution dissolve 1.32 gram of arsenious oxide in 100 cc. of concentrated hydrochloric acid and dilute to 1 liter. This contains 1 mg. of arsenic per cc.

#### ARSENIC BY ACID SODIUM THIOSULFATE

Addition of acid sodium thiosulfate solution, Bougault's reagent, to an arsenic solution causes reduction to metallic arsenic under proper conditions. The intensity of brown color is suitable for nephelometric estimation.<sup>108c</sup> The method will detect 0.002 mg. of arsenic. Comparisons must be carried out before precipitation can occur. The Pulfrich photometer has been used for the purpose, reading the results in terms of a standard glass and obtaining final results from a calibration curve.

**Procedure**—Transfer 5 cc. of sample solution free from organic mat-

<sup>108c</sup> A. Amati, *Biochim. terap. sper.* 20, 523-30 (1933).

ter to a tube and 5 cc. of standard <sup>103d</sup> to another tube. To each add 10 cc. of the reagent, prepared by dissolving 20 grams of sodium thiosulfate in 20 cc. of water, mixing with 200 cc. of concentrated hydrochloric acid and filtering. Heat in a boiling water bath for 30 minutes and compare.

#### ARSENIC BY MERCUROUS CHLORIDE

A qualitative test has been developed into a roughly quantitative method for estimation of arsenic <sup>103e</sup> and other elements. The coloration of a precipitate of mercurous chloride is compared. Gold, platinum, palladium, selenium, tellurium and iodine must be removed. Starch, dextrine, casein and blood do not interfere. Nitrates, per-salts, free halides, stannous chloride and hypophosphites must be absent. Copper must be less than 0.0003 gram and iron under 0.06 gram.

**Sample**—Add hydrochloric acid to the sample until 20 per cent of anhydrous acid is present. Add a substantial excess of mercurous chloride, warm and shake. Interfering elements are precipitated and arsenic remains in solution. Filter or decant and acidify to 30 per cent hydrochloric acid for use.

**Procedure**—Mix 0.1 gram of mercurous chloride with 5 cc. of 30 per cent hydrochloric acid previously boiled with mercurous chloride and allowed to stand in contact with it for 24 hours. Add sufficient of the sample to produce a positive coloration. Mix well and let settle. Compare the color of the precipitate with standards. The approximate colors obtained are as follows:

Mg. of Arsenic	Color on Mercurous Chloride
0.1	Deep, bright brown
0.01	Pinkish brown
0.001	Pink
0.0001	Cream
0.00002	Very slight cream

<sup>103d</sup> See p. 238.

<sup>103e</sup> Gordon G. Pierson, *Ind. Eng. Chem., Anal. Ed.* 6, 437-9 (1934).

## CHAPTER XXII

### ANTIMONY

#### ANTIMONY AS THE SULFIDE

ANTIMONY in copper or brass may be determined as an orange colloidal sulfide.<sup>1</sup> Bismuth does not interfere. The method has also been applied to organic samples. The error of the method is ordinarily less than 5 per cent. In the presence of large amounts of tin it may be 10 per cent or over. Gum arabic serves to render the colloidal sulfide more stable. Sulfur dioxide reduces antimony to the trivalent form.

**Sample**—*Brass, little or no tin present.* Dissolve 5 grams of sample in about 60 cc. of 1:1 nitric acid and 10 cc. of concentrated sulfuric acid. Evaporate to fumes. When cool dissolve in about 100 cc. of water. Add to the solution about 14 grams of solid sodium hypophosphite, cover and heat. When almost to boiling, remove and filter rapidly into a 750 cc. flask. Wash the copper sponge 2 or 3 times with hot water. Add about 2 grams of sodium hypophosphite and 100 cc. of concentrated hydrochloric acid to the filtrate. Boil for 15 minutes to precipitate arsenic. A large amount of arsenic may require further addition of sodium hypophosphite and additional boiling. Cool, add 10 cc. of benzene to coagulate colloidal arsenic, shake and filter through a wet filter into a 750 cc. flask. Wash the contents of the filter once or twice with hot water. Heat the filtrate to boiling. If more arsenic separates add more sodium hypophosphite and repeat the treatment.

Clean a strip of copper foil about 15 by 1.5 cm. coiled in a flat spiral, by warming in 1:1 nitric acid, wash with water and drop into the boiling solution of antimony. Boil from 1½ to 2 hours to plate out the antimony. Pour off the solution, wash the strip quickly with cold water, place in a small beaker and cover with water. Add about 1 gram of sodium peroxide immediately and warm until the deposit is dissolved. Pour off the solution and rinse the copper coil with cold water. The strip will be stained with cupric oxide. Immerse in 1:1 hydrochloric acid to dissolve this

B. S. Evans, *Analyst* 47, 1 (1922).

oxide. Any residual stains are antimony. If any remain repeat the treatment with sodium peroxide.

Add about 0.5 gram of zinc sulfide to the solution and let stand about 2 hours to remove any residual copper. Filter and wash the residue 2 or 3 times with cold water. The filtrate is the sample for determination.

*Brass, moderate amounts of tin present.* Proceed as usual until taken to sulfur trioxide fumes and dissolve in cold water. Add about 5 grams of potassium bitartrate and then 14 grams of sodium hypophosphite. Proceed as with small amounts of tin. Precipitation of copper will be more difficult.

*Organic Matter.*<sup>2</sup> Add magnesium oxide to the sample in a silica dish until its reaction is basic. Cover with a saturated solution of magnesium nitrate at the rate of 35–40 cc. per 100 grams of sample. Heat on a sand bath with stirring until the charred mass has begun to whiten. Crush with a pestle and continue heating until all the carbon has been consumed. If difficulty is encountered, let the ash cool, moisten with 5 cc. of saturated ammonium nitrate solution and again heat.

Moisten the carbon-free ash with 5 cc. of water and add 1:1 hydrochloric acid until the reaction is just acid to litmus. Dilute to about 20 cc. and saturate with hydrogen sulfide to precipitate the antimony. Filter and wash.

Dissolve the antimony sulfide from the filter with 2 cc. of concentrated hydrochloric acid. Dilute to about 15 cc. and filter again if necessary. Wash the filter well, add 1 cc. of 5 per cent gum arabic solution for each 100 cc. of final volume and dilute to a suitable concentration for colorimetric estimation. Take an aliquot of this solution as sample for development of color.

**Procedure**—Make the sample solution just acid with hydrochloric acid, dilute to about 80 cc., pass in sulfur dioxide a minute or two and boil down to about 10 cc. Place 5 cc. of a standard solution containing 0.1 mg. of antimony per cc. in a flask, add 80 cc. of water and a few drops of concentrated hydrochloric acid, pass in sulfur dioxide and boil down to 10 cc. Cool the flasks, add 5 cc. of a 1 per cent solution of gum arabic to each and dilute to 100 cc. Pass in hydrogen sulfide for a few seconds, place in Nessler tubes and compare the color by the dilution method. A blank should be run at the same time.

**Standard**—Dissolve 0.2765 gram of tartar emetic, antimony potassium

<sup>2</sup> Frank Bamford, *Analyst* 59, 101-2 (1934).



tartrate, in water, add 100 cc. of concentrated hydrochloric acid and dilute with water to 1 liter. One cc. corresponds to 0.1 mg. of antimony.

#### ANTIMONY BY REACTION WITH PYRIDINE AND AN IODIDE

Antimony in high grade tin may be separated by the Reinsch reaction and determined by the golden yellow color formed with pyridine and an iodide in acid solution.<sup>3</sup> As little as 0.05 mg. of antimony may be estimated by this method. In a series containing 0.25 to 3 mg. of antimony the average error was 3 per cent. With 0.1–0.75 mg. of antimony added to 1 gram of tin containing arsenic and bismuth the error was 7 per cent.

Hydrochloric acid causes a serious reduction of color and large amounts of alkali chlorides entirely bleach it. A slight loss of antimony results in evaporating to sulfur trioxide fumes to remove chlorides, unless nitric acid is added in slight excess of the chlorides present. With weak acids like acetic the reaction does not take place. Reasonable amounts of tin and arsenic give no color with the reagent. Bismuth and several heavy metals give colored precipitates. Zinc gives a white crystalline precipitate so that the zinc sulfide method of separation of antimony from bismuth and copper cannot be used. In the reaction pentavalent antimony is evidently reduced to the trivalent form which reacts.

**Sample—Alloys.** If 0.05 per cent of antimony is present take 1 gram of sample, if less, 5 grams. Dissolve in 50 cc. of concentrated hydrochloric acid with addition of excess bromine in small amounts, while solution is taking place. Add 10 grams of oxalic acid and 350 cc. of water. Boil gently and add about 0.5 gram of sodium hypophosphite to reduce free bromine and render the solution colorless. Prepare a copper spiral, plate out antimony and remove with sodium peroxide as in the preceding method.<sup>4</sup>

Precipitate copper sulfide from the alkaline solution with hydrogen sulfide, warm about 15 minutes to coagulate, and filter. Wash with 1 per cent ammonium nitrate solution. To the filtrate add 5 cc. of concentrated sulfuric acid and evaporate to fumes, adding a few drops of nitric acid near the end of the evaporation. Cool and dissolve in about 15 cc. of water. A clear solution should result.

*Alternative Method.*<sup>5,6</sup> The method of separation of antimony by

<sup>3</sup> S. G. Clarke, *Analyst* 53, 373-9 (1928).

<sup>4</sup> See p. 251.

<sup>5</sup> A. A. Vasil'ev and M. E. Shub, *J. Applied Chem. (U.S.S.R.)* 6, 560-2 (1933).  
ow Park, *Ind. Eng. Chem., Anal. Ed.* 6, 189-90 (1934).

precipitation with manganese dioxide is also a very satisfactory one. For details see separation of bismuth from copper by the same procedure.<sup>7</sup>

**Procedure—Direct Comparison.** To a 100 cc. Nessler tube add 10 cc. of 1 per cent gum arabic solution, then 5 cc. of 20 per cent potassium iodide solution. Follow with 1 cc. of a 10 per cent aqueous solution of pyridine and 1 cc. of a solution of sulfur dioxide prepared by 1:10 dilution of a saturated solution. To these add 60 cc. of 1:3 sulfuric acid and the solution of the sample. The above order of addition must be followed. Transfer the sample with about 5 cc. of water, and dilute to volume. Stir with a glass rod to develop the color.

As a parallel standard add the above reagents except that 80 cc. of 1:3 sulfuric acid are used. Add antimony solution containing 0.1 mg. per cc.<sup>9</sup> until the color is duplicated, adjusting the volumes as usual and comparing through the length of the tubes.

If more than 10 cc. of standard are required the color will be too deep for accurate comparison and turbidity may result. In that case take a 20 cc. aliquot of the colored solution being compared and add to the same reagents in another tube. The intense color will be reduced, the turbidity will disappear and the color can be duplicated with a fresh standard.

**Extraction.** If the antimony content is very low, extract the aqueous solutions of sample and standard developed for direct comparison of color, with 10 cc. of amyl alcohol.<sup>5</sup> Repeat the extractions with 5 cc. of amyl alcohol. Combine the extracts from the sample and from the standard and compare by balancing.

#### ANTIMONY BY PHOSPHOMOLYBDOTUNGSTIC ACID

The reaction of trivalent antimony with phosphomolybdotungstic acid may be used for its colorimetric estimation.<sup>10</sup> The method will determine 0.05 mg. of antimony in 100 cc. of solution.

**Procedure—**Reduce the antimony in acid solution by addition of sodium sulfite. Boil to remove all excess sulfur dioxide. Adjust the acidity of the solution to 0.02–0.1 *N*. For this  $\beta$ -dinitrophenol is a suitable indicator. Add Folin's reagent,<sup>11</sup> phosphomolybdotungstic acid, acidified

<sup>7</sup> See p. 214.

, *J. Soc. Chem. Ind. Japan* 34, Suppl. Binding 322-3 (1931).

<sup>11</sup> See p. 252.

to less than 0.1 *N*, until a suitable color is developed. The amount of reagent required varies with the antimony content. Heat the solution to develop the full color.

Compare with a standard containing the same amount of reagent at the same acidity, heated at the same time.

## CHAPTER XXIII

### TIN

#### TIN BY AMMONIUM MOLYBDATE

ammonium molybdate, which is quantitatively

The color is a molybdenum blue complex of variable composition if the concentration of tin is over 30 mg. per liter but, with controlled conditions, of fixed composition under that concentration. The color develops over a period of 30 minutes and after reaching its maximum intensity it fades for at least 90 minutes.

As little as 0.1 mg. of tin can be detected. Oxidizing and reducing agents must be absent. Antimony gives a faint greenish color which will not be confused with the blue of tin but may make readings difficult. Arsenic and zinc do not interfere. Concentration of the sample by precipitation on manganese dioxide has been suggested.<sup>2</sup>

minute.

6 cc. of acid ammonium molybdate of the usual strength,<sup>3</sup> 3 cc. of 2 *N* sodium hydroxide solution. Compare after 30 minutes with a similar solution prepared from a standard stannous chloride solution at the same time. As Beer's law holds, comparison may be by dilution or balancing. Duplication is not possible and a series of temporary standards is unnecessarily difficult of preparation. The use of a permanent series of standards of color mixtures of Army and Ring<sup>4</sup> is suggested by Huttig.

**Standard**—Prepare the standard solution by dissolving 1 gram of pure tin in 100 cc. of 1:1 hydrochloric acid and diluting to 1 liter with

---

<sup>1</sup> G. F. Huttig, *Chem.-Ztg.* 47, 341 (1923).

<sup>2</sup> Bartholow Park, *Ind. Eng. Chem., Anal. Ed.* 6, 189-90 (1934).

<sup>3</sup> See p. 522.

<sup>4</sup> H. V. Army and C. H. Ring, *J. Ind. Eng. Chem.* 8, 309 (1916).

the same acid. This standard contains 1 mg. of tin per cc. For preparation of the standard color solution for comparison, dilute a definite volume of this standard to 30 cc. with 1:1 hydrochloric acid, heat to boiling and treat, beginning with addition of zinc, as specified for the sample.

#### TIN AS THE SULFIDE

As another of the sulfide methods, tin may be so determined in the absence of interfering ions.<sup>5</sup> The comparisons are of colloidal sulfides. Stannous sulfide is unsuitable for the purpose but by suitable manipulation both stannous and stannic ions may be estimated.

Since it is necessary to make a preliminary oxidation with bromine and some excess remains, this oxidizes some of the hydrogen sulfide to free sulfur. The error so introduced is not great but does tend toward high results. This may be partially counterbalanced by selecting the standard next in brightness below that which on first comparison matches the sample. This correction does not apply to the solutions where bromine has not been added. Amounts as small as 1-5 mg. per 100 cc. can be satisfactorily estimated by this method.

**Procedure—Total Tin.** To 10 cc. of sample add bromine-water drop by drop until a permanent pale yellow is obtained. This oxidizes stannous ion to stannic ion. Mix with 5 cc. of a saturated solution of hydrogen sulfide and allow to stand for 1 hour to complete formation of the colloidal sulfide. Compare with a series of standard suspensions prepared in the same way from a standard stannic salt solution.

**Stannic Tin.** Mix 5 cc. of sample with 5 cc. of saturated aqueous solution of hydrogen sulfide and let stand for 1 hour without undue exposure to air. Add 5 cc. of concentrated hydrochloric acid. The stannous sulfide goes into solution at once and the effect of the acid on the stannic sulfide after it has been completely formed is so slight that it may be neglected. Compare the color with that of a series of known stannic sulfide suspensions. Multiply the value by 2, to be comparable with the determination of total tin which used double the amount of sample.

**Stannous Tin.** Subtract the stannic tin from the total tin to give the stannous tin content.

**Standard**—Dissolve 1 gram of tin in 100 cc. of concentrated hydrochloric acid and dilute to about 200 cc. Add bromine water drop by drop until a permanent pale yellow color is developed. Dilute to 1 liter. This

<sup>5</sup> Rudolf Hanssen, *Chem.-Ztg.* 54, 143 (1930).

dilution of 10 cc. to 100 cc.

### TIN BY CACOTHELINE

presence of 30 in w  
acid the  
h reaction sulfuric acid lowers the  
sensitivity. In hyd acid the color develops in 15 minutes; in  
water 3 times as long i The color fades after a few hours. Anti-  
mony also gives a color the reagent but only above 0.01 *M*. Bisulfite.  
hydrosulfite, sulfite, titanous, mercuric, chromate, chromic and nitrate  
ions interfere. Other ions such as molybdate, which react with the tin  
must also be absent. Colored ions in sufficient concentrations interfere,  
such as cobalt, copper, iron, nickel and vanadium.

**Reagent**—Dissolve 39.4 grams of dry brucine in 200 cc. of 5 *N* nitric acid in the cold, then warm to 50–60° for 15 minutes. The red solution becomes reddish yellow and crystals of cacotheline separate out. Let stand in ice-water for a few hours to complete the crystallization. Filter by suction and wash the crystalline material with *N* nitric acid, acetone and ether. The yield should be 86–89 per cent of theoretical, or about 44–45 grams.

**Procedure**—To 10 cc. of the sample solution add 0.1 cc. of a 0.25 per cent aqueous solution of cacotheline. Mix and let stand until the maximum color develops. Compare with a suitable standard of the same acidity as the sample, similarly treated at the same time. If desired to develop greater sensitivity and hasten the development of color, add 1 cc. of concentrated hydrochloric acid to sample and standard before adding the reagent.

Leuchs and Friedrich Leuchs, *Ber.* 43, 1042-51 (1910).

Leuchs and Hans Kaehn, *Ber.* 55, 724-32 (1922).

Leuchs, Bernhard Winkler and W. Robert Leuchs, *Ann.* 55, 3936-50

55, (1922).

(1929).

and Lewis

*J.* 7, 26-7 (1935).

## CHAPTER XXIV

### ALUMINUM

#### ALUMINUM BY ALIZARIN-S

By the use of the sodium salt of alizarin monosulfonic acid as little as 0.1 p.p.m. of aluminum may be detected in water.<sup>1,1a</sup> The aluminum salt of the dye is formed, giving a yellow to red color in acid solution. The presence of moderate amounts of calcium, magnesium or zinc salts has no effect on the results.

Iron, chromium, cobalt and manganese must be removed if more than a trace is present.<sup>2</sup> Copper present must be considerably less than 1 mg. One mg. of sulfur as sulfide, 1 mg. of stannic tin, 10 mg. of nitrate ion or 10 mg. of nickel may be present in 50 cc. of solution. The range of the method is 0.0004 to 0.2 mg. of aluminum in a 50 cc. Nessler tube, or 0.005 to 5 mg. in a Kennicott-Campbell-Hurley colorimeter. Starch glycerite is an effective stabilizer unless heat is used during lake formation.<sup>3</sup> This method has frequently been criticized; other work<sup>2</sup> indicates that it is a satisfactory method. The final comparison should be made at pH 4-5 without an excess of hydrochloric acid, acetic acid, or ammonium chloride.<sup>4</sup> Citric acid should not be present. It has been applied to aluminum in biological material by ashing and using the solution of ash in 10 per cent sulfuric acid.<sup>5</sup>

**Sample—General.** Dissolve a sample containing 0.005 to 0.05 mg. of aluminum oxide in 5 to 20 cc. of 1:10 hydrochloric or sulfuric acid. If the sample exceeds these limits, take an aliquot. Avoid the use of nitric acid as it causes the color to fade more than other acids do, although all cause a slow fading.

<sup>1</sup> F. W. Atack, *J. Soc. Chem. Ind.* 34, 936 (1915).

<sup>1a</sup> D. S. Savchenko, *Zavodskaya Lab.* 1933, No. 2, 23-5; *Chimie & Industrie* 31, 1375 (1933).

<sup>2</sup> J. H. Yoe and W. L. Hill, *J. Am. Chem. Soc.* 50, 748 (1928).

<sup>3</sup> Walter E. Thrun, *Ind. Eng. Chem., Anal. Ed.* 2, 8-9 (1930).

<sup>4</sup> A. P. Musakin, *Zavodskaya Lab.* 3, 1085-9 (1934).

<sup>5</sup> F. P. Underhill and F. I. Peterman, *Am. J. Physiol.* 90, 1-14 (1929).

*oxide in metallic aluminum.*<sup>5a</sup> Dissolve 1 gram of sample of aluminum in 2 cc. of 5 per cent mercuric nitrate solution and 70 cc. of 5 per cent tartaric acid solution. Filter and wash the filter until mercury is completely removed. Ash the paper containing the aluminum oxide in platinum and fuse with a small amount of sodium carbonate mixture. Dissolve the melt in about 50 cc. of water. Pass through a fine filter. Acidify the solution with acetic acid. Use as sample.

**Procedure**—To the solution in a Nessler tube add 5 cc. of *N* hydrochloric acid, 1 cc. of alizarin-S solution, and water to make 10 cc.

stirring

50 cc.

tion treated similarly.

**Standards**—A series of standards is not satisfactory. The method of choice is the method of the question because it takes some time for the color to develop after the standard comes into contact with the reagent. Prepare a standard at the same time as the sample and subject to the same conditions of temperature and acidity. Radiate the standard in each. As a solution for preparing standards, dissolve 0.9286 gram of alum,  $K_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24H_2O$ , in water and dilute to 1 liter. Pipet out 10 cc. of this solution and dilute again to 1 liter. The resulting solution contains 0.001 mg. of aluminum oxide per cc.

#### BY FORMATION OF A LAKE WITH AURIN TRICARBOXYLIC ACID

The action of aurin tricarboxylic acid with aluminum salts to form a bright red lake is the basis of a sensitive estimation of aluminum.<sup>6</sup> The reagent is also called Aluminon. The aluminum lake is formed in the presence of an acetic acid-acetate buffer. This solution may be made alkaline with ammonium hydroxide without decomposing the lake, although it does not form in an alkaline solution. By means of this property the interference of chromium is prevented, as the chromium complexing the aluminum compound in appearance, forms in acetate solution and is decolorized on the addition of ammonium hydroxide.

<sup>5a</sup> V. P. Okhotin and N. Zubareva, *Zavodskaya Lab.* 2, No. 6, 18-19 (1933).

<sup>6</sup> L. P. Hammett and C. T. Sottery, *J. Am. Chem. Soc.* 47, 142 (1925).



Under the conditions adopted, bismuth, lead, antimony, stannic, mercuric and titanium ions and silicic acid give white precipitates; cadmium, zinc, manganese, cobalt and nickel ions do not precipitate, and reasonable amounts of chromium, alkaline earths and phosphorus do not interfere in the presence of ammonium carbonate. Iron must be removed. The hydroxides or basic acetates of beryllium, yttrium, lanthanum, cerium, neodymium, erbium, zirconium and thorium all give deeper red lakes than aluminum. All but beryllium are decolorized by ammonium carbonate.<sup>8</sup> Scandium gives a red lake insoluble in ammonium hydroxide but soluble in ammonium carbonate. Gallium forms a lake more slowly, which is more like that of aluminum. Indium gives a red color stable to ammonia. Germanium does not react.<sup>9</sup> Small amounts of sulfur dioxide and hydrogen sulfide may be present if the color is compared immediately.<sup>10</sup>

The method cannot be applied to amounts of aluminum greater than 1 mg. unless starch or gum arabic<sup>3</sup> is added, because of precipitation, and is preferably applied to 0.1 to 0.5 mg. An amount as small as 0.05 mg. may be estimated and 0.002 mg. detected. It is essential that Pyrex or other ware free from aluminum be used. A blank should be obtained on the reagents by an analysis with an aluminum-free sample.

The intensity of color increases with decreasing pH to 4.0, and the lake precipitates at pH 1.8.<sup>12</sup> Increased concentration of reagent or increased temperature also increase the color. The maximum intensity is obtained by heating to 80° for 10 minutes or boiling for 1 minute. About 64 per cent of the maximum color ordinarily develops at room temperature in 15 minutes; the maximum is not reached in 60 hours. By sufficient increase of dye concentration, the maximum can be reached at room temperature in 24 hours. Optimum conditions were reported in 25 cc. of solution containing 5 cc. of 5 *N* ammonium acetate at pH 4.5. The color of the dye itself must be destroyed. The pH at which this occurs is influenced by the amount of ammonium chloride or ammonium acetate present. The intensity of the color lake is decreased above pH 7.3, but not up to that pH.

The method was found applicable to estimation of aluminum removed from cooking vessels by the foods cooked in them.<sup>13</sup> It has been applied

<sup>8</sup> A. R. Middleton, *J. Amer. Chem. Soc.* 48, 2125 (1926).

<sup>9</sup> Robert B. Corey and H. W. Rogers, *J. Amer. Chem. Soc.* 49, 216-7 (1927).

<sup>10</sup> G. E. F. Lundell and H. B. Knowles, *J. Ind. Eng. Chem.* 18, 60 (1926).

<sup>12</sup> O. B. Winter, W. E. Thrun and O. D. Bird, *J. Am. Chem. Soc.* 51, 2721-31 (1929).

<sup>13</sup> C. A. Dunbar Mitchell, *J. Roy. Army Med. Corps* 61, 99-107, 193-201 (1933).

to ash from organic materials<sup>12</sup> and the procedure developed has been further refined.<sup>15</sup> Comparison at a buffered pH of 6.3 has also been recommended.<sup>16</sup> Some natural coloring matters can be similarly used. An extract of marigold leaves gives a yellow color, extract of buckthorn berries a blue.<sup>16a</sup>

**Sample**—*Brass, Bronze, Phosphor-Bronze, Lead-Base, and Tin-Base Bearing Metals.* Transfer 1 gram of alloy to a 250 cc. flask and dissolve in 5 cc. of concentrated nitric acid. Add 30 cc. of 8 per cent sodium hydroxide solution and heat to boiling, rotating over a free flame. Prepare a sodium sulfide solution by saturating 20 cc. of 8 per cent sodium hydroxide solution with hydrogen sulfide and adding 20 cc. of the same sodium hydroxide solution. Add 20 cc. of this sodium sulfide solution to the solution of the sample, swirl for a few minutes and filter.

Acidify the filtrate with 1:1 hydrochloric acid and add 2 cc. in excess. Digest at 40–60° until the precipitate settles. Filter and boil the filtrate until all hydrogen sulfide is expelled. If suspended sulfur is present, clarify by small additions of nitric acid. Evaporate to 20–30 cc. and filter if necessary. Dilute to a known volume.

*Spelter, Brass, Phosphor-Bronze, Lead-Base and Tin-Base Bearing Metals.* Dissolve the alloy as in the previous method, add 50 cc. of 8 per cent sodium hydroxide solution and boil for 1 minute. Add the following amounts of the special sodium sulfide solution: 0.5 cc. for tin-base bearing metal; 1 cc. for spelter, cast bronze and phosphor-bronze; 2 cc. for brass and journal bearings, and 4 cc. for lead-base bearing metal. Swirl for a few minutes, filter and continue as above. If a precipitate is formed at the time of production of the color it should be white and this will not interfere.

*Tissue by Dry Ashing.*<sup>15</sup> Select a sample of 5–150 grams of well washed tissue or equivalent sample. Dry it over night in a platinum dish at 110°. Place on a triangle in a cold muffle and heat electrically at such a rate that the temperature is raised to a faint red heat in about 8 hours. Continue at that heat over night, with a slow stream of oxygen passing into the muffle furnace to complete the ashing.

Add 10–25 cc. of concentrated hydrochloric acid, according to the

---

<sup>15</sup> Gerald J. Cox, E. W. Schwartze, Raymond M. Hann and Richard B. Unangst, *Ind. Eng. Chem.* 24, 403-5 (1932).

<sup>16</sup> Paul S. Roller, *J. Am. Chem. Soc.* 55, 2437-8 (1933).

<sup>16a</sup> A. I. Potapov, *Compt. rend. aca. sci. U. R. S. S. 1*, 589-91 (1934).

nature of the ash, and 25 cc. of water. Evaporate to dryness to dehydrate silica. Add 10 cc. of 1:3 hydrochloric acid and 25 cc. of water to the ash and boil for 5-10 minutes. Transfer to a tube and centrifuge at 1800 r.p.m. for 5 minutes. Decant the solution into a 100 cc. Erlenmeyer flask.

If the residue is carbon-free, discard it. If not, transfer to a platinum crucible and dry. Volatilize the silica with hydrofluoric and sulfuric acids and ignite until free of acid and carbon. Fuse in a 1:1 mixture of sodium and potassium carbonates and dissolve in the sample solution.

Add 1 cc. of concentrated nitric acid and 1 cc. of 6 per cent ferric sulfate solution to the sample solution. Evaporate to about 10 cc. and dilute to about 60 cc. Add 5 cc. of monosodium phosphate and 2 cc. of 0.04 per cent bromphenol blue solutions. Add 1:1 ammonium hydroxide until a permanent precipitate is obtained. Add 40 per cent sodium acetate solution until the pH is adjusted to about 4.2. This is necessary for quantitative separation from calcium phosphate. Centrifuge for 5 minutes at 1800 r.p.m. and discard the upper layer.

Dissolve the precipitate of iron and aluminum phosphates in 5 cc. of 1:1 hydrochloric acid and 1.25 cc. of glacial acetic acid. Add 15 cc. of hot water. Add 5 cc. of 25 per cent sodium hydroxide solution and let stand for 1 hour with frequent stirring. Wash the rod, and centrifuge for 5 minutes to throw down the ferric hydroxide.

Prepare a double 9 cm. filter paper by washing on the filter with warm 5 per cent sodium hydroxide solution and then with hot water until substantially all the alkali has been removed. Filter the sample solution through these papers into a 100 cc. volumetric flask containing 1 cc. of 1:1 hydrochloric acid. Wash the precipitate in the centrifuge tube once with 20 cc. of hot water and decant the washings through the filter. Do not transfer the precipitate to the paper. These filter papers remove the last traces of iron by adsorption to give an iron-free filtrate as shown by the thioglycolic acid test.<sup>19</sup> Wash the filter, cool the filtrate, acidify to litmus with 1:1 hydrochloric acid and dilute to 100 cc.

*Tissue by Wet Ashing.*<sup>20</sup> Wash the weighed tissue into a quartz flask with a mixture of 4 parts of concentrated hydrochloric acid and 5 parts of 60 per cent perchloric acid. Add sufficient acid to keep the contents semiliquid and warm gently until organic matter is destroyed. Evaporate the mixture almost to dryness, dissolve in 5 cc. of 1:20 hydrochloric acid and dilute to 15 cc. with water.

<sup>19</sup> Edward Lyons, *J. Am. Chem. Soc.* 49, 1916-20 (1927).

<sup>20</sup> H. Steudel, *Biochem. Z.* 253, 387-94 (1932).

Neutralize to methyl red in a centrifuge tube with 1:1 ammonium hydroxide and add 1 cc. of saturated ammonium acetate solution. Mix and heat in boiling water for 10 minutes. Centrifuge to remove the precipitate. Decant the upper layer and dissolve the precipitate in 1 cc. of 1:1 hydrochloric acid. Dilute to 15 cc. and add 1.25 cc. of glacial acetic acid and 5 cc. of 25 per cent sodium hydroxide solution. Mix and let the iron precipitate settle for 1 hour. Transfer the upper layer containing the

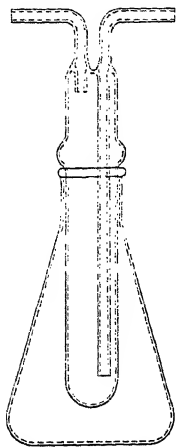
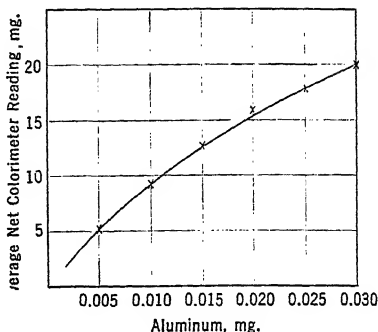


FIG. 76

Apparatus for Development of Aurin Tricarboxylic Acid Lake



Typical Calibration Curve of Aluminum Aurin Tricarboxylate Against Artificial Standard

aluminum as aluminate to a Nessler tube. Add 1.1 cc. of 1:1 hydrochloric acid and 0.75 cc. of glacial acetic acid and dilute to a suitable volume.

**Procedure**—Pipet 20 cc. of the sample into a dry 250 cc. glass-stoppered Erlenmeyer flask, designed for the purpose (Figure 76). Add 25 cc. of a reagent <sup>21</sup> containing 78 grams of ammonium acetate, 54 grams of ammonium chloride, 80 cc. of 0.1 per cent aurin tricarboxylic acid and 60 cc. of 1:1 hydrochloric acid per liter. At this point the pH of the sample should be between 4.5 and 5.5. Put the condenser in the neck of the flask and boil for 1 minute after steam bubbles first appear. Let cool for 1 minute and then cool with running water.

<sup>21</sup> Based on O. B. Winter, W. E. Thrun and O. D. Bird, *J. Am. Chem. Soc.* 51, 2721 (1929).

Add 1.8 per cent ammonium carbonate solution until the pH is raised to about 7.1. This will normally require 4.8–5.0 cc. Stopper the flask and shake up and down 20 times. This is carefully controlled for proper pH adjustment. Too little shaking will result in low pH and incomplete alteration of the dye to the yellow form. If the shaking is too vigorous or prolonged, the pH will be high and the lake color will fade. Cautiously loosen the stopper to release carbon dioxide and let the flask stand for 20 minutes with the stopper loosened to decolorize excess dye. The pH must now be between 7.0 and 7.3 and preferably at 7.1.

Compare in a colorimeter by balancing as described under Standard.

**Standard—Natural.** A natural standard may be developed at the same time as the sample and used for comparison.

**Artificial.**<sup>22</sup> Mix 5 cc. of 0.04 per cent thymol blue solution and 8 cc. of 1:1 hydrochloric acid with water and dilute to 500 cc. Calibrate this against natural standards, prepared with the same reagents, set at a depth of 30 mm. Prepare a calibration curve. This will be correct only for the same lots of reagents. It must be recalibrated when new stocks are obtained as it automatically corrects for the blank on the reagents. The pink color of the standard is stable for 6 months. While it does not match the color of the lake exactly, readings are accurate to  $\pm 0.1$  mm. of depth.

#### AURIN TRICARBOXYLIC ACID IN WATER ANALYSIS

This reagent has also been used by a modified procedure for the determination of aluminum in water.<sup>23</sup>

**Procedure—***In the presence of iron when the latter has already been determined.* To 100 or 200 cc. of sample water add 1 or 2 cc. of 4 *N* hydrochloric acid, evaporate to dryness on the water bath and ignite the residue at a dull red heat for a few minutes. Cool, take up with a few drops of 4 *N* hydrochloric acid and 5 cc. of hot water and filter off the silica. Wash with small portions of hot water and neutralize the filtrate, testing with litmus paper, by adding 4 *N* hydrochloric acid. Transfer to a 50 cc. Nessler tube. Add 5 cc. of *N* hydrochloric acid, 5 cc. of 3 *N* ammonium acetate solution and dilute to 30 cc. Add 5 cc. of a 0.1 per cent solution of the reagent and mix. After 5 minutes add 5 cc. of 5 *N* ammonium hydroxide and 10 cc. of 5 *N* ammonium carbonate solution slowly

<sup>22</sup> W. E. Thrun, *J. Phys. Chem.* 33, 977 (1929).

<sup>23</sup> J. H. Yoe and W. L. Hill, *J. Am. Chem. Soc.* 49, 2395 (1927).

with stirring. Dilute to 50 cc. and mix. After 20 minutes compare with a standard solution of aluminum treated in the same way but to which an amount of iron equivalent to that found in the sample by previous determination has been added.

*Aluminum separated from iron.* Evaporate from 300 to 500 cc. of the sample of water in platinum. When nearly dry add 1 cc. of 1:1 hydrochloric acid and, after moistening the sides of the dish, evaporate to dryness. If much organic matter is present ignite the residue at a dull red heat. Moisten with 1:3 hydrochloric acid, dissolve in hot water and filter off the silica, washing the latter with hot water.

Heat the filtrate and washings to boiling, oxidize with concentrated nitric acid or bromine and concentrate to about 100 cc. Precipitate the hydroxides of aluminum and iron by adding 5 *N* ammonium hydroxide until the solution is just neutral to methyl red. Filter while hot. Dissolve in 5 cc. of warm 5 *N* nitric acid and wash the filter with small quantities of hot water. Evaporate the filtrate to 1 cc., make alkaline with 6 *N* sodium hydroxide solution, heat to boiling and filter through a very small paper. Wash the filter with small quantities of hot water and neutralize the filtrate with hydrochloric acid by testing with litmus paper. Transfer to a 50 cc. Nessler tube and let cool. Treat with reagents and complete as above except that comparison is made with a standard solution of aluminum to which no iron has been added.

#### ALUMINUM BY HEMATOXYLIN

The hematoxylin method is very satisfactory for aluminum in filtered water<sup>24</sup> and in soil extracts<sup>25</sup> when used in a buffer solution of ammonium carbonate and then acidified. If iron is present in a concentration greater than 10 mg. per liter, an equal amount must be added to the standard.<sup>26</sup> Iron is destroyed when the solution is acidified. Iron in water do not

interfere with the determination of the sample followed by neutralization is a wise precaution since not all aluminum all being in the form of aluminum ion.<sup>26a</sup> The color after adding ammonium carbonate solution, pH 8.2, is lavender-blue. After acidifying with acetic acid, pH 4.5, it is yellowish-brown.

The color shown by the reagent is solely from aluminum ion. The reagent is stable for 2 to 3 weeks. This method gives results accurate to

<sup>24</sup> W. D. Hatfield, *Ind. Eng. Chem.* 16, 233 (1924).

<sup>25</sup> J. L. Steenkamp, *J. S. African Chem. Inst.* 13, 64-70 (1930).

<sup>26</sup> E. Naumann, *Chem.-Ztg.* 57, 315-16 (1933).

<sup>26a</sup> M. A. Shpak and Shubaev, *Iskusstvennoe Volukno* 6, No. 1, 51-2

0.1 p.p.m. of aluminum. It checks with the gravimetric method if the pH is over 6.0 and is more sensitive than the latter near the two extremes of the aluminum hydroxide insolubility zone, pH 5.7 to 7.3.

**Procedure**—To 50 cc. of water sample in a tall Nessler tube add 1 cc. of saturated ammonium carbonate solution and 1 cc. of 0.1 per cent hematoxylin solution made by dissolving the white crystals in boiling water. Mix by inverting twice. Let stand 15 minutes for the maximum lavender color to form, then acidify with 1 cc. of 30 per cent acetic acid. Mix and compare the color with standard color tubes prepared in the same way at the same time from a standard ammonium alum solution and distilled water, containing from 0.0 to 1.0 p.p.m. of aluminum. Not over 15 minutes difference in time of preparation of the tubes is permissible. If less than 0.15 p.p.m. of aluminum is present compare against white paper through the length of the tubes, but with higher concentrations, compare through the sides of the tubes.

**Standard**—As standard dissolve 0.8366 gram of ammonium alum in distilled water, and dilute to 1 liter. Each cc. of this contains 0.05 mg. of aluminum. For the series dilute 0.0–1.0 cc. in 0.1 cc. stages to 50 cc. with distilled water. These contain 0–1 p.p.m. of aluminum.

If the sample of water before treatment does not compare with distilled water, standard raw water may be filtered through a Berkefeld filter and used for preparation of standards to compensate for naturally occurring ions.

#### ALUMINUM BY 8-HYDROXYQUINOLINE BY CONVERSION TO A DYE

An aluminum salt reacts with 8-hydroxyquinoline. When this precipitate is coupled with a diazo compound, the resulting arylazo dye in alkaline solution has a deep yellow-red color suitable for colorimetric estimation.<sup>27</sup> As little as 0.025 mg. of aluminum can be estimated. Urotropine can be used to remove calcium and magnesium, and the usual methods for removal of iron and phosphate. Iron causes a darker color, calcium and magnesium low results. The method is recommended for use with absolute methods of measurement of color. With 0.01 mg. of aluminum, errors as high as 20 per cent may occur, but with upward of 0.025 mg. the error should not be over 4 per cent.

<sup>27</sup> F. Alten, H. Weiland and H. Loofmann, *Angew. Chem.* 46, 668-9 (1933).

**Sample**—Measure out 2–5 cc. of acid solution of the sample which may contain 0.02 to 0.5 mg. of aluminum. This solution may also contain calcium, magnesium and iron. Transfer to a 10 cc. centrifuge tube and add a few crystals of ammonium sulfate. When these are dissolved, add 1 cc. of a 30 per cent solution of urotropine. Heat to boiling for 1 minute over a small flame and centrifuge for 3 minutes at 2000 r.p.m. For removal of the supernatant liquid a capillary tube with the tip turned up is recommended. Remove the supernatant liquid and add 2 cc. of water. Stir well and heat to boiling. Centrifuge and remove the wash water. The precipitate so obtained contains the aluminum and iron.

Add 1 cc. of 0.5 *N* sodium hydroxide solution to the precipitate. If more than 0.05 mg. of aluminum is present also add 1 cc. of water. Heat to boiling and centrifuge. Separate the upper layer, which contains the aluminum, and transfer to a round-bottom centrifuge tube. Wash the iron residue twice with 1 cc. portions of hot water, heating to boiling with each. The total volume of aluminate solution and washings to be used as sample should be 3–4 cc.

**Reagent**—Dissolve 0.3226 gram of pure 8-hydroxyquinoline in 10 cc. of glacial acetic acid and dilute to 1 liter with water. Each cc. of this will react with 20 mg. of aluminum.

**Procedure**—Acidify the iron-free sample with 2 drops of glacial acetic acid and add 0.6 cc. of a saturated solution of sodium acetate. Add 0.5–1 cc. of the reagent. If more than 0.05 mg. of aluminum is present, precipitation occurs quickly. For smaller amounts let stand over night. Heat the liquid and precipitate at 70° for one-half hour. Centrifuge and remove the supernatant liquid. Wash the sides of the tube and the precipitate twice with 1 cc. portions of hot water. Leave the stirring rod or a hollow capillary in the tube. Add 2 cc. of a mixture of equal volumes of 2 *N* hydrochloric acid and 95 per cent alcohol. Heat in hot water until the precipitate is completely dissolved and transfer the solution to a 50 cc. flask. Wash the tube 3 times with water. If more than 0.05 mg. of aluminum is present, dilute to volume and take a suitable aliquot.

To the solution, or an aliquot, add a mixture of 0.5 cc. of a solution of 8.6 grams of sulfanilic acid in 1 liter of 30 per cent acetic acid and 0.5 cc. of sodium nitrite solution containing 2.85 grams per liter. Mix and let stand for 10 minutes. Add 10 cc. of 2 *N* sodium hydroxide solu-



## BY CUPFERRON

tion and dilute to 50 cc. At the end of 10 minutes, compare with a standard which has been similarly treated.

### ALUMINUM BY CUPFERRON

By adding cupferron, the ammonium salt of nitrosophenylhydroxylamine, to a neutral or faintly acid solution of aluminum salt, a colloidal solution yellow to transmitted light and blueish to reflected light, is obtained with a concentration below 3 mg. of aluminum per liter.<sup>28</sup> The method is applicable in the presence of any substance not precipitated by cupferron in faintly acid solution in sufficient dilution, such as magnesium, silver, divalent mercury, pentavalent antimony, tri- and pentavalent arsenic, lead, cadmium, zinc, manganese, nickel, cobalt and chromium. Trivalent chromium ion begins to produce turbidity at a concentration of about 5 mg. per liter. The last four of the series give colored solutions and in their presence aluminum can only be determined nephelometrically. Copper, tin, trivalent antimony, bismuth and iron interfere as they also give very slightly soluble derivatives with cupferron. The procedure differs slightly according to the concentration of aluminum.

The method has been used with an average error of less than 1 per cent and a maximum error of 3.2 per cent.<sup>28</sup> Beer's law holds very well so that standard and sample may be quite different in concentration of test ion. When compared colorimetrically, addition of 3 g. of zinc sulfate to 0.003 g. of aluminum gave an error of less than 2 per cent. Compared nephelometrically, results in the presence of 2 g. of nickel nitrate were equally accurate.

The method is probably applicable to other ions forming difficulty soluble compounds with cupferron such as copper, tin, trivalent antimony, bismuth and iron.

**Procedure**—*From 3 to 30 mg. of aluminum per liter.* Add to 25 cc. of unknown, 1 cc. of a freshly prepared 5 per cent aqueous solution of cupferron and 1 cc. of a 0.1 per cent solution of gelatin. Add the same quantities of reagents to 25 cc. of a solution containing a suitable amount of standard and compare in the colorimeter, preferably by superimposing a piece of blue glass between the eye and the ocular to increase the sensitivity.

*From 1.5 to 3 mg. of aluminum per liter.* Proceed as above but omit the gelatin solution.

<sup>28</sup> L. de Brouckère and E. Belcke, *Bull. soc. chim. Belg.* 36, 288 (1927).

*From 0.15 to 1.5 mg. of aluminum per liter.* Add to 25 cc. of sample 1 cc. of a freshly prepared 1 per cent aqueous solution of cupferron. Compare with 25 cc. of a standard solution treated with the same amount of reagent, using a nephelometer with lateral illumination.

**Standard**—Dissolve 0.2460 gram of aluminum sulfate,  $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ , in 1 liter of 0.002 *N* sulfuric acid. This corresponds to 0.01 gram of aluminum per liter or 0.01 mg. per cc. This may be further diluted with 0.002 *N* sulfuric acid according to the concentration needed.

#### ALUMINUM BY PHOSPHOMOLYBDOTUNGSTIC ACID

Aluminum can be precipitated with hydroxyquinoline and that precipitate redissolved and estimated by Folin's reagent.<sup>30</sup> Zinc and bismuth must be absent.

**Procedure**—Acidify 1–4 cc. of the solution of sample to about pH 3.0. Add 0.5 cc. of saturated sodium acetate solution and 4 drops of 0.5 per cent solution of oxine (hydroxyquinoline) acetate. Heat on a water bath 15 minutes to cause the precipitate to settle. When cool dilute to about 5 cc. and centrifuge for 10 minutes at 2000–2500 r.p.m. Decant the clear liquid. Stir up the precipitate with 5 cc. of water and centrifuge again. Repeat with 2 cc. of water.

Dissolve the precipitate in 1 cc. of 1:2 hydrochloric acid and rinse into a 25 cc. volumetric flask with 15 cc. of water. Add 1 cc. of Folin's reagent<sup>31</sup> and 6 cc. of cold saturated sodium carbonate solution. Dilute to volume and compare after 30 minutes with a standard similarly prepared at the same time.

#### ALUMINUM BY QUINALIZARINE

In faintly acid solution aluminum forms a violet-purple lake with quinalizarine, 1, 2, 5, 8-hydroxyanthraquinone.<sup>32</sup> In alkaline solution beryllium gives a blue color with the same reagent. The aluminum color varies from intensely violet for 1 mg. of aluminum per liter to faintly violet for 0.1 mg. per liter. Copper and iron are objectionable. Tin, antimony, and bismuth give precipitates at the hydrogen-ion concentra-

<sup>30</sup> M. Teitelbaum, *Z. anal. Chem.* **82**, 366-74 (1930).

<sup>31</sup> See p. 183.

<sup>32</sup> I. M. Kolthoff, *Chem. Weekblad* **24**, 447 (1927); *J. Am. Phar. Assoc.* **17**, 260-1 (1928).

tion used, but are prevented from interfering by the addition of sodium tartrate or Rochelle salt.

**Buffer Solution**—Mix 10 parts of 5 *N* acetic acid with 9 parts of 5 *N* ammonium hydroxide. On diluting tenfold with the sample solution this gives a pH between 5.4 and 5.8.

**Procedure**—To 10 cc. of a neutral solution of aluminum add 0.25 to 1 cc. of ammonium acetate-acetic acid buffer solution. Add 0.3 cc. of a 0.1 per cent alcoholic solution of quinalizarine. Shake and compare after 15 or 30 minutes with a standard solution of aluminum similarly treated.

#### ALUMINUM BY HYDROXYMETHYLANTHRAQUINONE

The purple color of the aluminum salt of hydroxymethylanthraquinone in faintly acid solution is a sensitive test for traces of aluminum.<sup>33</sup> Interference by copper is avoided by addition of sodium thiosulfate. Iron, tin, bismuth and antimony do not interfere if Rochelle salt or sodium tartrate are added, but the sensitivity is diminished by these salts.

**Procedure**—The procedure is identical with that of the preceding method.

#### ALUMINUM BY ERIOCHROME CYANINE

Eriochrome cyanine, one of the triphenylmethane dyes, is orange red, which becomes violet red in the presence of aluminum.<sup>33a</sup> The color is also altered by nickel, zinc, manganese, chromium and iron in the presence of aluminum.<sup>33b</sup> The effect of iron or manganese is seven times that of aluminum, that of magnesium double that of aluminum. Phosphate and organic materials decrease the color. It is also influenced by pH. Interfering substances can be removed or their effect corrected. The method is accurate to 6 per cent in the presence of substantial amounts of iron or manganese, and to better than that in their absence.

**Sample**—Select a sample containing 0.01–0.001 mg. of aluminum. If organic matter is present, ash carefully in platinum. Add about 0.5 cc.

<sup>33</sup> I. M. Kolthoff, *J. Amer. Pharm. Assoc.* 17, 360-1 (1928).

<sup>33a</sup> F. Alten, W. Weiland and E. Knippenberg, *Z. anal. Chem.* 96, 91-8 (1934).

<sup>33b</sup> F. Alten, B. Wandrowski and E. Hille, *Angew. Chem.* 48, 273-5 (1935).

of concentrated sulfuric acid to the carbon-free ash and heat to sulfur trioxide fumes to convert the aluminum to sulfate.

If silicates are present in the ash, add 1 gram of a 1:1 mixture of sodium and potassium carbonates and fuse. After keeping liquid for 10–15 minutes, cool and dissolve as much as possible in 2 cc. of water. Filter and treat the residue with three 2 cc. portions of 1:4 nitric acid. Transfer this solution to a quartz flask and boil to drive off carbon dioxide. To precipitate any phosphates present, prepare an ammonium molybdate solution by dissolving 150 grams of ammonium molybdate in water and, after dilution to 1 liter, pouring into 1 liter of 1:1 nitric acid.<sup>33c</sup>

Transfer the sample solution to a test tube and dilute to about 8 cc. Introduce the standard and blank at this point, add to each 2 cc. of 0.1 per cent monopotassium phosphate solution, and dilute to about 8 cc. Add drop by drop without stirring 2 cc. of the ammonium molybdate solution to sample, standard and blank. Shake after 1 hour, and after 10 minutes add 2 cc. of 50 per cent ammonium nitrate solution. Shake and after another hour filter each into a 20 cc. quartz test tube. Wash each filter with 2 cc. of 2 per cent ammonium nitrate solution. Some small amount of ammonium molybdate remains unprecipitated, but it is the same in each case.

Add 2 cc. of a solution of 5 grams of uranyl acetate in 100 cc. of 5 per cent acetic acid to each solution. Add 2 drops of bromothymol blue indicator solution and 1:3 ammonium hydroxide drop by drop with stirring until the indicator becomes greenish, and, following that, a yellowish white precipitate of molybdic acid and ammonium uranate is obtained. The aluminum is carried down with these precipitates. Let stand for 2 hours and centrifuge. Decant the upper layer and dissolve the residue in 10 cc. of 5 per cent sodium carbonate solution. If definite brown precipitates of iron and manganese remain, again centrifuge and decant the liquids into 100 cc. volumetric flasks. Wash the residues with 2 cc. of water, centrifuge and add the liquids to the flasks. Some aluminum will still be held by the gelatinous precipitates. Dissolve the precipitates in 2 cc. portions of 1:20 hydrochloric acid and dilute to 5 cc. Add 2 cc. of 2 *N* sodium hydroxide solution to each and boil. Very little aluminum is adsorbed by this precipitate. Cool, centrifuge and decant these liquids into the flasks.

If there is little precipitate after solution in sodium carbonate solu-

---

<sup>33c</sup>J. König, "Die Untersuchung landwirtschaftlich und gewerblich wichtiger Stoffe," 3rd ed., p. 963. Paul Parey, Berlin (1906).

tion, transfer the solutions as a whole to the flasks and omit the other steps of separation of aluminum from iron and manganese.

*Acetate Buffer*—Dissolve 154 grams of ammonium acetate, 109 grams of sodium acetate trihydrate and 6 grams of glacial acetic acid in water and dilute to 1 liter.

**Procedure**—Shake the flasks of sample, standard and blank to eliminate carbon dioxide and add 15 cc. of 0.1 per cent eriochrome cyanine solution. Add 2 *N* sodium hydroxide solution until the orange-red color changes to a dark violet red. A precipitate of ammonium uranate and molybdic acid results. Add 5 per cent hydrochloric acid until this precipitate just redissolves, leaving only a faint turbidity. Add 20 cc. of the acetate buffer solution and dilute to 100 cc. Compare with the standard, which has been similarly treated, within 1 hour. Use a yellow-green filter for the comparison. The color is usually stable for at least 2 hours longer.

## CHAPTER XXV

### CHROMIUM

CHROMIUM in solution may be determined as either the monochromate or the dichromate. Great care should be taken to duplicate the conditions under which comparisons are made as the color is influenced by hydrolysis, hydration, temperature, the degree of acidity or alkalinity, and particularly the concentration of the solution.<sup>1</sup> In solutions more dilute than 0.003 per cent the color of chromates and dichromates is identical. Higher and equal concentrations of chromic acid and chromates vary until the color of chromic acid becomes 2 to 3 times as great as that of a dichromate. Very dilute solutions of all chromates contain only monochromate molecules and ions. More concentrated solutions contain dichromates and at still greater concentrations, or in acid, or when heated, dichromates contain trichromates.

#### CHROMIUM AS THE CHROMATE

In this method chromium is converted to the chromate by alkaline fusion and compared in alkaline solution with solutions prepared from the salt. Interference by manganese is prevented by addition of hydrogen peroxide or alcohol as reducing agent.

If potassium or sodium nitrate is added in the fusion and the crucible is attacked, a yellow color due to platinum may be present. For that reason, either entirely avoid nitrate in the fusion, or at the most use not over 5 per cent based on the amount of carbonate used.

**Sample—Iron, Steel or Brass.**<sup>2</sup> Dissolve 5 grams of iron, steel, or brass in a flask with 50 cc. of 6 *N* hydrochloric acid. Nearly neutralize with a 60 per cent sodium carbonate solution, complete with a 10 per cent suspension of barium carbonate and add 10 cc. excess of barium carbonate suspension. Boil gently 10–15 minutes with a watch glass covering the flask to prevent oxidation of the iron. Filter rapidly and wash the

---

<sup>1</sup> W. M. Dehn, *J. Am. Chem. Soc.* 36, 829 (1914).

<sup>2</sup> W. T. Hall and R. S. Williams, "Examination of Iron, Steel and Brass," 150. McGraw-Hill & Co., New York (1921).

precipitate twice with hot water. Transfer the filter paper and contents to a platinum crucible. Burn off the paper carefully and fuse the ash with a mixture of 5 grams of sodium carbonate and 0.25 gram of potassium nitrate. Dissolve the fusion in water, transfer to a beaker and add 2 cc. of 3 per cent hydrogen peroxide to reduce any permanganate formed. Boil a few minutes to decompose excess hydrogen peroxide and filter.

*Rocks and Ores.*<sup>3</sup> If over 0.2 per cent is present use a 1 gram sample; if less, use 2-5 grams. Fuse the finely ground sample with sodium carbonate and extract the fusion with water. Add a few drops of alcohol to destroy the color from sodium manganate and filter. If the yellow color is very faint concentrate by evaporation.

If too dilute to concentrate by evaporation add 10 per cent mercurous nitrate solution to the cold, slightly alkaline filtrate. If a very large precipitate forms add nitric acid carefully to reduce the alkalinity. Heat, filter, and dry the precipitate. Remove the latter from the filter paper, and ignite in a platinum crucible. Fuse the residue with a little sodium carbonate, leach with water, and filter to obtain the solution for comparison.

*Rocks and Soils containing Manganese.*<sup>4</sup> The separation of manganese and iron is described<sup>5</sup> under the determination of manganese. Precipitate silver from the filtrate by the addition of sodium chloride solution. Evaporate to a suitable volume. The yellow color is that of sodium chromate. At least 0.2 per cent chromic oxide must be present for determination from the 1 gram sample used.

*Fabric.*<sup>6</sup> Ash a sample of fabric of suitable size and fuse the ash 4-5 minutes with 0.5 gram of equal parts of potassium chlorate and potassium carbonate. Cool, dissolve in a small volume of water, and filter if necessary. Add sufficient sodium carbonate to produce a clear yellow color. Dilute to a volume which gives a color suitable for comparison.

**Procedure**—Place the solution of sample in a Nessler tube and duplicate the color by addition of standard sodium chromate solution to an equal volume of distilled water containing 5 grams of sodium carbonate.

A known volume of standard may also be diluted to match the color of the sample, or a series of standards prepared.

<sup>3</sup> W. F. Hillebrand, *J. Am. Chem. Soc.* 20, 454-60 (1898); *U. S. Geol. Survey, Bull.* 176, 80 (1900).

<sup>4</sup> M. Sittrich, *Z. anorg. Chem.* 80, 171-3 (1913).

<sup>5</sup> See p. 335.

<sup>6</sup> F. W. Richardson, W. Mann, and N. Hanson, *J. Soc. Chem. Ind.* 22, 614 (1903).

*Alternative Procedures.* The intensity of yellow has been measured with the Lovibond tintometer and chromium determined by comparison with a suitable table.<sup>6</sup> Rough checks have also been obtained by rendering sample and standard acid and adding potassium iodide and starch solution. Comparison was by dilution.

**Standard**—If results are to be reported as chromium dissolve 6.580 grams of sodium chromate,  $\text{Na}_2\text{CrO}_4 \cdot 10\text{H}_2\text{O}$ , in distilled water, make alkaline with 1 gram of sodium carbonate, and dilute to 1 liter. Each cc. is equivalent to 1 mg. of chromium. By further dilution standards of 0.1 mg. or 0.01 mg. per cc. are prepared.

If results are to be reported as chromic oxide,  $\text{Cr}_2\text{O}_3$ , dissolve 5.1050 grams of sodium chromate in water, make alkaline with 1 gram of sodium carbonate, and dilute to 1 liter. Each cc. is equivalent to 2 mg. of the oxide.

#### CHROMIUM AS THE DICHROMATE

Chromium in steel has been estimated as the dichromate. Below 0.003 per cent, the color of chromate and dichromate is identical because only chromate ions are present.<sup>1</sup> Iron must be removed by extraction with ether<sup>9</sup> or by combination with phosphate.<sup>10</sup> Only the latter method is given here. The phosphate prevents precipitation of oxides of iron which would carry down basic chromate. Molybdenum, vanadium and tungsten do not interfere.

**Sample**—Heat 4 grams of sample with 30 cc. of 1:3 sulfuric acid and 20 cc. of water until dissolved, adding more water if ferrous sulfate crystals separate. Add 5 to 10 cc. of nitric acid and boil until the fumes have disappeared. Add about 25 grams of ammonium phosphate, 250 cc. of water, and more nitric acid if ferric phosphate precipitates. Heat to boiling, add a saturated solution of potassium permanganate a few drops at a time, until an excess is present, then 12 drops more and continue boiling for 15 minutes.

Place 120 cc. of a 20 per cent solution of sodium hydroxide in a large beaker, add about 14 drops of a saturated solution of potassium permanganate and boil for some minutes. Add more permanganate if a green color appears. Add 10 cc. of a 5 per cent solution of manganese

<sup>6</sup> A. A. Blair, "The Chemical Analysis of Iron," 8th ed., p. 177. J. B. Lippincott Co., Philadelphia, Pa. (1918).

<sup>10</sup> B. S. Evans, *Analyst* 46, 38, 285 (1921).



sulfate to destroy the permanganate. Pour in the acid solution of sample slowly. Transfer to a 500 cc. volumetric flask and let cool. Test to see that the solution is alkaline. Add 10 cc. of glacial acetic acid. Test to make sure that the solution is now acid. Dilute to volume, shake, and let settle. Filter through dry paper, rejecting the first 20 cc.

**Procedure—*Nickel and Cobalt Absent.*** In the absence of nickel and cobalt place 100 cc. of the filtrate in a comparison tube and about 90 cc. of distilled water in a second tube. To each add 20 cc. of 1:3 sulfuric acid and run standard potassium dichromate into the blank tube until the two match.

***Nickel and Cobalt Present.*** In the presence of nickel or cobalt transfer 100 cc. of filtrate to a flask, heat to boiling, and add sodium hydroxide solution until a precipitate separates, avoiding a large excess. Cool, filter into a comparison tube, add 20 cc. of 1:3 sulfuric acid and complete as above.

**Standard—**As a standard solution dissolve 0.283 gram of carefully dried potassium dichromate in water and dilute to 1 liter. Each cc. is equivalent to 0.1 mg. of chromium.

#### CHROMIUM AS CHROMIC ION

The color of chromic ion from iron or steel may be used for its estimation by comparison with a standard of similar composition similarly treated.<sup>10a</sup> Best results are obtained with 16–24 mg. of chromium in 50 cc. of 1:8 sulfuric acid. The interference of nickel and vanadium can readily be corrected for by having similar amounts in the standard. Any loss due to retention by the undissolved carbon may be expected to be similar in sample and standard.

**Procedure—**Transfer a 2 gram sample to a flask. In another flask take 2 grams of standard. To each add 35 cc. of 1:8 sulfuric acid. Insert a funnel in the neck of the flask and heat until solution is complete, adding water from time to time. Cool quickly and filter. Wash the carbon on the filter with small amounts of water and dilute the filtrates to 50 cc. Compare.

<sup>10a</sup> E. Fogel'son, *Zavodskaya Lab.* 2, No. 9, 33-6 (1933).

## CHROMIUM IN TANNING LIQUORS

One-bath chrome liquors may be examined colorimetrically after filtration by comparing with a stock liquor conveniently diluted or with a spent liquor which has been analyzed for chromium.<sup>11</sup> The basicity,  $\text{Cr}_2\text{O}_3/\text{H}_2\text{SO}_4$ , varies only from 0.87 before use to 0.75 after use, so that the method is reasonably accurate.

The method is not applicable to 2-bath liquors because of the variable amount of reduced chromium salts present with dichromates. Reduction of all of the chromium to chromic chloride is not satisfactory because of difficulty in getting a clear color and because of the time required.

## CHROMIUM BY DISODIUM-1,8-DIHYDROXYNAPHTHALENE-3,6-DISULFONATE

The analysis of steel for small amounts of chromium has been simplified by use of the above reagent. The resultant color is pink and is so delicate that 0.001 per cent of chromium can be determined in a 2 gram sample. There is no advantage in using the method when more than 0.6 per cent of chromium is present. If a considerable quantity of vanadium is present this produces a brown color which is apt to obscure the results.<sup>12</sup> If the vanadium present is less than the chromium a correction may be introduced. Subtract from the percentage of chromium shown, one third of the percentage of vanadium as shown by a separate analysis in order to obtain the true percentage of chromium present. If the ratio of chromium to vanadium is high the error may be negligible and so can be disregarded. A correction also may be introduced by using a similar amount of vanadium in the standard.

**Procedure**—For steels containing 0.15 per cent of chromium or less, use a 0.4 gram sample; for more use a 0.2 gram sample. Dissolve the sample in 10 cc. of 1:3 sulfuric acid. Add 0.5 cc. of concentrated nitric acid to oxidize the iron. Evaporate to sulfur trioxide fumes. The presence of excess nitric acid interferes with the color. Cool, dilute with 50 cc. of water and add 50 cc. of a 10 per cent solution of sodium hydroxide and 1 gram of sodium peroxide. Boil for 5 minutes to destroy the excess of peroxide, cool, dilute to 200 cc. in a volumetric flask and filter. Use as a sample 100 cc. of filtrate. Add 2 cc. of 85 per cent phosphoric acid and 8 cc. of concentrated sulfuric acid.

<sup>11</sup> J. T. Wood and D. J. Law, *J. Soc. Chem. Ind.* 29, 398 (1910); *J. Am. Leather Chem. Assoc.* 5, 295-7 (1910).

<sup>12</sup> F. Garratt, *J. Ind. Eng. Chem.* 5, 298 (1913).

The final solution should not contain more than 20 per cent of sulfuric acid by volume. Add 2 cc. of a 1 per cent aqueous solution of disodium-1,8-dihydroxynaphthalene-3,6-disulfonate. A pink to cherry red color develops and may be compared after 15 minutes.

The comparison may be made by the dilution method only, because of the peculiar character of the color developed and the danger of interference.

**Standard**—The standard selected is a steel of known chromium content, treated by the same method as the sample and at the same time. As an alternative, a known amount of potassium dichromate may be added to the solution of a chromium-free steel. A suitable final dilution is such that 1 cc. contains 0.001 gram of chromium.

Some chromium is retained by the precipitate but at least 95 per cent is present in the final solution. Any green of ferric ion is destroyed by the phosphoric acid present. Tungsten and molybdenum do not interfere. Titanium is removed in the determination. The average error is less than 1 per cent.

**Application to Analysis of Plant Ash**—The same method has also been applied to determination of chromium in plant ash.

Ash 5 grams of dried plant matter. Dissolve the ash in 40 cc. of water, add 1 cc. of syrupy phosphoric acid, and dilute to 50 cc. Remove 10 cc. and add 1 cc. of a 0.1 per cent solution of the reagent.<sup>13</sup> After standing 15 minutes compare the color with that of a chromium-free plant ash to which was added a standard solution of chromate or dichromate. This standard should be treated with the reagent at the same time. Compare again on the following day.

#### CHROMIUM BY DIPHENYLCARBAZIDE

This reaction was qualitatively reported for chromium in strongly acid solution with acetic or hydrochloric acid.<sup>14</sup> The intense violet color is sensitive to 1 p.p.m. Copper and iron give a similar color in neutral solution but not in the presence of hydrochloric or acetic acid. In the presence of large amounts of iron 0.004–0.0017 per cent of chromium was found, accurate to 0.0001 per cent.<sup>15,15a</sup> It has been applied to chromium

<sup>13</sup> P. Koenig, *Chem.-Ztg.* 35, 277 (1911).

<sup>14</sup> A. Cazeneuve, *Analyst* 25, 331 (1900); *Bull. soc. chim. Paris* [3] 25, 761.

<sup>15</sup> B. S. Evans, *Analyst* 46, 285 (1921).

<sup>15a</sup> A. Moulin, *Bull. soc. chim. Paris* [3] 31, 295-6.

in air as collected by a cotton plug of a suction apparatus,<sup>16,16a</sup> and to chromium in tumors.<sup>16b,16c</sup> In the latter case photometric measurement rather than colorimetric estimation was used. Interference by zinc, cobalt, nickel, lead, silver and gold is suggested.

**Sample—General.** The sample should be free from organic matter, as prepared by one of the preceding methods, and taken to fumes with sulfuric acid. The sulfuric acid content should be about 10 per cent.

To 50 cc. of sample add 50 cc. of 10 per cent sodium hydroxide solution and 1 gram of sodium peroxide. Boil for 5 minutes or longer if necessary to destroy the excess of peroxide. Cool and dilute to 200 cc. in a volumetric flask. Mix well and filter, protecting from evaporation.

**Tumors.** Ash a sample of tissue in a platinum crucible. Fuse the ash with 50 mg. of sodium carbonate and 10 mg. of potassium chlorate. Dissolve the cooled melt in water. Add 0.2 cc. of 1:4 hydrochloric acid and warm to drive off carbon dioxide. Then dilute to 10 cc. Use the entire volume or an aliquot as sample.

**Procedure—**To 10 cc. of sample solution add 0.2 cc. of reagent containing 0.5 gram of diphenylcarbazide dissolved in 10 cc. of glacial acetic acid and diluted to 100 cc. with 95 per cent alcohol. Let stand 20 minutes for development of the maximum color. Compare with a standard similarly treated at the same time.

#### CHROMIUM BY AFTERCHROMING DYED WOOL

Serichrome Blue R<sup>17,18</sup> dyes wool to a bright crimson with a faint bluish tinge. Commercially, subsequent treatment with chromic acid converts this to a fast navy blue color. The treatment of wool dyed with

---

<sup>16</sup> M. E. Loginov, *Leningrad Inst. Hig. Truda Tekh. Bezoposnosti Trudui i Materialui Byull.* Nos. 7-8, 42-4 (1931).

<sup>16a</sup> Yu. D. Gol'denberg, *Zavodskaya Lab.* 3, 506-7 (1934).

<sup>16b</sup> Andrew Dingwall and Hal T. Beans, *Am. J. Cancer* 16, 1499-1501 (1932).

<sup>16c</sup> Andrew Dingwall, R. G. Croser and Hal T. Beans, *Am. J. Cancer*, 21, 606-11 (1934).

<sup>17</sup> V. Friedländer, *Fortschr. Theerfarbenfabrikation ver. Ind.* 3, 791-3 (1896).

<sup>18</sup> G. Schultz, "*Farbenstofftabellen*," Dye No. 164 (1914).

Serichrome Blue R with very dilute chromic acid solutions develops a further bluish tint which may be used for quantitative estimation by comparison with a series of prepared standards.<sup>19</sup>

Good checks were obtained when chromium was introduced in various forms. If desired, the red color may be stripped from the treated wool with 0.1 *N* sodium carbonate solution. In so doing the blue color of chromed Serichrome Blue R is not affected. That the resulting color is characteristic of chromic acid is indicated by the absence of similar results from molybdic, tungstic, vanadic and permanganic acids, ferrous sulfate, manganous sulfate and chrome alum.

**Reagent**—The reagent in this case is dyed wool. Add 40 cc. of water, 0.1 gram of sodium sulfate and 0.02 gram of sulfuric acid to a 125 cc. Erlenmeyer flask. Add 2 grams of wool flock and shake to insure thorough wetting of the fibers. Add 20 cc. of a 0.5 per cent solution of Serichrome Blue R. Stir well and heat on a steam bath for 30 minutes. Filter on a Büchner funnel, wash until the washings show no acid, and dry.

**Sample**—Precipitate iron, aluminum and chromium from the sample solution by careful addition of a slight excess of 1:1 ammonium hydroxide. Heat to boiling to coagulate and filter on an asbestos mat in a Gooch crucible. Wash the precipitate with distilled water.

Transfer the asbestos carrying the precipitate to a beaker. Add 30 cc. of water and 3 cc. of *N* sodium hydroxide solution. Mix well and add 5 cc. of 3 per cent hydrogen peroxide solution. Let stand for 30 minutes and heat to boiling until the excess hydrogen peroxide is decomposed. Filter by suction. Add 6 cc. of approximately *N* sulfuric acid.

**Procedure**—Add 0.1 gram of dyed wool to the acidified solution containing the dichromate derived from the sample. Heat on a steam bath for 30 minutes. Filter by suction and dry. Unless the quantity of chromium is so great as to exceed the maximum of the series of standards, the chromium content can be read off from the colored wool.

<sup>19</sup> G. C. Spencer, *Ind. Eng. Chem., Anal. Ed.* 4, 245-6 (1932).

**Standards**—Dissolve 0.282 gram of potassium dichromate in water and dilute to 100 cc. Each cc. contains 1 mg. of chromium. For use dilute 10 cc. of this stock solution to 1 liter. Each cc. now contains 0.01 mg. of chromium.

To each of a series of 10 beakers add 50 cc. of water, 3 cc. of approximately *N* sulfuric acid and 0.1 gram of the dyed wool. Stir to wet the fiber thoroughly. Add 1 to 10 cc. of the standard dichromate solution to the series of beakers. Cover with watch glasses and heat on a steam bath for 30 minutes with occasional stirring. Filter on a small Büchner funnel, wash and dry. Mount on a suitable white background with nitrocellulose or other suitable adhesive. The blue shade of the series of samples corresponds to the varying amounts of chromic acid present.

## CHAPTER XXVI

### IRON

#### IRON BY THIOCYANATE

THE thiocyanate method for determining iron depends on the oxidation of iron to the ferric condition after which the reaction between ferric ion and thiocyanate ion is used to produce a red color proportional to the amount of iron present.<sup>1</sup> The color is affected by the hydrogen-ion concentration of sample and standard. The determination can be made more delicate by extracting the iron compound with ether or amyl alcohol, or preferably with a mixture of 5 volumes of amyl alcohol to 2 volumes of ether. This is due to the undissociated salt not ionizing in the nonpolar solvent. Such extraction is advisable for amounts of 5 p.p.m. or less. The disagreeable odor can be avoided by substituting monobutyl ether for the amyl alcohol, mixing with ether as usual. The extracted color is stated to be more intense and not to fade for 24 hours.<sup>2</sup>

In the presence of titanium, yellow compounds, such as  $K_2TiO(CNS)_4$ ,  $H_2O$  and  $K_3Ti(CNS)_3 \cdot 6H_2O$ , are formed. These are extracted by amyl alcohol-ether mixtures, but not by ether alone.<sup>3</sup> The color is bleached by sunlight<sup>4</sup> but this can be prevented by addition of an oxidizing agent such as nitric acid.<sup>5</sup> By drying an ether solution of ferric thiocyanate the color has been intensified.<sup>6</sup> By addition of acetone the color is also intensified, the method made more sensitive,<sup>7</sup> and error due to phosphate prevented.<sup>8</sup> Peroxides cause a yellowish color, but as a micro method, extraction of acidified blood containing thiocyanate with a peroxide ether, consisting of 1 cc. of 0.03 per cent hydrogen peroxide to 50 cc. of ether, has been proposed.<sup>9</sup> This oxidizes the iron and carries out the extraction in a single operation.

<sup>1</sup> H. Ossian, *Pharm. Centr.* 13, 205 (1837).

<sup>2</sup> Adolph Bernhard and I. J. Drechter, *Science* 75, 517 (1932).

<sup>3</sup> Kurt Heise, *Farbe u. Lack* 1931, 464.

<sup>4</sup> H. S. Pulsifer, *J. Am. Chem. Soc.* 26, 967-75 (1904).

<sup>5</sup> L. Szegoe and B. Cassoni, *Giorn. chim. ind. applicata* 15, 281-3 (1933).

<sup>6</sup> H. W. van Urk, *Pharm. Weekblad.* 63, 1101-7 (1926).

<sup>7</sup> W. McKim Marriott and C. G. L. Wolf, *J. Biol. Chem.* 1, 451-61 (1905-6).

<sup>8</sup> F. S. Fowweather, *Biochem. J.* 20, 93-8 (1926).

<sup>9</sup> Georg Barkan, *Klin. Wochschr.* 11, 598-9 (1932).

The color is of the undissociated ferric thiocyanate which has been stated to have various compositions. The ionized salt has been reported colorless.<sup>10</sup> More recent work<sup>11</sup> indicates that in aqueous solution the color is due to  $[\text{Fe}(\text{CNS})_6]^\equiv$  and in nonpolar solution to  $\text{Fe}[\text{Fe}(\text{CNS})_6]$ . The red compound may hydrolyze to an insoluble brown basic compound.<sup>12</sup> The colored compound is light-sensitive. The pH of the solution must be carefully controlled.<sup>13</sup>

Addition of sodium sulfate lowers the color but hydrochloric acid will bring it back. For water 4 milliequivalents per 100 cc. are desirable; in the presence of sodium sulfate this must be increased, and if acetates are present a greater amount is required, as expected. Chlorides and nitrates do not affect the color. The photoelectric colorimeter has been used for reading the color.<sup>13a</sup>

The method has had extensive use, having been applied to such diverse materials as commercial glucose, blood, tissue, milk, urine, lead carbonate, cement, battery acid, water and various metallic oxides. A thiocyanate method has been found satisfactory for meat and bone scrap.<sup>14</sup> It is the most rapid and accurate method for estimation of iron in organic materials.<sup>15</sup> Among limiting amounts which can be detected<sup>13</sup> are 0.000007 per cent in commercial sodium sulfate, 0.00002 per cent in water in the absence of hydrochloric acid and 0.000005 per cent in its presence, and 0.01 mg. per cc. in a solution of plant ash.<sup>17</sup> Many substances interfere, such as silver, copper, fluorides, pyrophosphates, arsenates, oxalates, citrates, tartrates, iodates, to a lesser extent acetates and sulfates, and in some cases cobalt. The effect of orthophosphates is not serious up to several hundred times the amount of iron present.<sup>18</sup> Because of possible interference and because the color is not proportional to the concentration, the sample and standard should have nearly identical composition and concentration. One method of isolating the iron is to precipitate ferric hydroxide together with hydrated manganese peroxide, the latter being soluble and colorless under the conditions for color comparison.

<sup>10</sup> H. N. Stokes and J. R. Cain, *J. Am. Chem. Soc.* 29, 409 (1907).

<sup>11</sup> H. L. Schlesinger and H. B. Van Valkenburgh, *J. Am. Chem. Soc.* 53, 1212-16 (1931).

<sup>12</sup> J. F. Sacher, *Farben-Chem.* 2, 120-2 (1931).

<sup>13</sup> H. W. van Urk, *Chem. Weekblad* 25, 703-6 (1928).

<sup>13a</sup> M. Bendig and H. Hirschmüller, *Z. anal. Chem.* 92, 1-7 (1933).

<sup>14</sup> G. S. Fraps and J. F. Fudge, *J. Assoc. Official Agr. Chem.* 15, 307-10 (1932).

<sup>15</sup> G. Dominici, *Folia Clinica* 3, 65-82 (1928).

<sup>17</sup> W. Scholz, *Z. Pflanzenernähr., Düngung Bodenk.* 26A, 212-16 (1932).

<sup>18</sup> Geoffrey W. Leeper, *Analyst* 55, 370-1 (1930).



Ferric sulfide may be precipitated similarly with cadmium sulfide as a carrier.

Extreme care as to the purity of the reagents is necessary. The methods of purification are the usual ones. Many reagents tested were found to contain iron.<sup>19</sup> A blank test should show at most only a very faint pink. Correcting for a blank determination lowers the accuracy of the determination. Hydrofluoric acid showed 0.007 mg. of iron per 10 cc. and hydrochloric acid up to 0.004 mg. Extreme care to avoid contamination from stoppers and apparatus is necessary. Cleaning with a mixture of hydrochloric acid and thiocyanate is advisable.

If nitric acid is used and is not entirely free from nitrous acid a false result will be obtained. This can be avoided by addition of hydrogen peroxide to oxidize nitrous acid to nitric.<sup>20</sup>

Thiocyanic acid is preferable as a reagent to a thiocyanate because of the ease with which it can be purified from iron. Fading of color is prevented by the presence of potassium persulfate in the presence of mercuric thiocyanate.

**Sample**—The sample is preferably not over 1 gram. If less than 5 p.p.m. of iron are present, a larger sample is necessary. In such a case, a method of separation of the bulk of the other ions present is usually required.

*Silica or Soluble Silicates.*<sup>19</sup> Treat a sample of 0.5 gram of silica or 1.5 gram of silicate with 10 cc. of hydrofluoric acid and 1 cc. of nitric acid. Take up the residue with 5 cc. of 1 : 1 nitric acid and dilute to 45 cc.

As standard, treat 0.5 gram of iron-free silica in the same way. An alternative is to treat a similar sample, as standard, and extract the iron with ether until it no longer gives a test with thiocyanate. A known amount of iron is then added by the duplication method.

*Clay.*<sup>22</sup> Fuse a 0.05 gram sample with 5 grams of potassium bisulfate. Take up the fusion in water and dilute to a suitable volume.

Compare by duplication with a standard containing 0.05 gram of pure aluminum oxide fused with 5 grams of potassium bisulfate and diluted to approximately the same volume as the sample. The ether-amyl alcohol method is recommended. Results can be obtained in 20 minutes.

*Portland Cement.* Dissolve 0.1 gram of cement in 5 cc. of hydrochloric acid and 5 cc. of nitric acid and filter. As a solution for the standard

<sup>19</sup> H. L. Smith and J. H. Cooke, *Analyst* 51, 503-10 (1926).

<sup>20</sup> W. B. Walker, *Analyst* 50, 279-83 (1925).

<sup>22</sup> J. W. Mellor, *Trans. Ceramic Soc. (England)* 8, 125-31 (1910).

dissolve 0.1 gram of cement in which the ferric oxide has been carefully determined in 5 cc. of hydrochloric acid and 5 cc. of nitric acid and filter. Compare by dilution or balancing if sample and standard do not vary by more than 10 per cent.

*Lead and Lead Compounds.*<sup>23,24</sup> In the case of pig lead, lead oxides or lead carbonate, use 30 grams of refined or 10 grams of crude product. Weigh the finely divided sample into a 400 cc. beaker and add small portions of hot 1:1 nitric acid until completely dissolved. If basic lead nitrate is formed, dilute slightly with water and boil. To red lead also add 30 to 40 cc. of 3 per cent hydrogen peroxide or 5 grams of sodium sulfite and boil until lead peroxide is dissolved.

Add with stirring 30 cc. of 1:1 sulfuric acid. Filter by decantation, finally washing the precipitate on the paper. Neutralize the filtrate with concentrated ammonium hydroxide, adding 4 cc. in excess. Boil, filter and wash. Copper may be determined in the filtrate. Dissolve the precipitate with 5 cc. of 1:1 hydrochloric acid and put into a 250 cc. volumetric flask. Wash the filter paper free from acid, dilute to the mark and mix. Place 10 cc. in a comparison tube and add 3 drops of concentrated nitric acid. Dilute to 95 cc. As a standard add 5 cc. of 1:1 hydrochloric acid and 3 drops of concentrated nitric acid to distilled water and dilute to 95 cc. Compare by duplication.

If considerable bismuth is present a yellow instead of red color will result.<sup>25,26</sup> As a specific example, 2.0 mg. of bismuth were found to interfere with the estimation of 0.5 mg. of iron. To avoid interference, after precipitation of lead nearly neutralize the solution and precipitate bismuth with hydrogen sulfide. The precipitated bismuth sulfide does not carry down iron.

The addition of 5-10 cc. of concentrated hydrochloric acid to the sample per 10 grams of lead is also stated to cause disappearance of the yellow color and result in satisfactory determination of the iron content. In that case add an equal volume to the standard.

*Lead Compounds. Alternative Method.*<sup>27</sup> Precipitation of lead as the sulfate has been stated to give low results. An alternative procedure follows, designed for red lead.

Mix a 10 gram sample with 25 cc. of a cold saturated hydrazine hydro-

<sup>23</sup> John A. Schaeffer, *J. Ind. Eng. Chem.* 4, 659 (1912).

<sup>24</sup> B. S. White, *Ibid.* 7, 1935-6 (1915).

<sup>25</sup> H. Heinrichs and M. Hertrich, *Glastech. Ber.* 2, 112-5 (1924).

<sup>26</sup> H. Heinrichs, *Ibid.* 5, 351 (1927); *J. Soc. Glass Tech.* 12, 182 (1928).

<sup>27</sup> H. Heinrichs, *Z. angew. Chem.* 41, 450-3 (1928).

chloride solution. Add 50 cc. of concentrated hydrochloric acid and boil for 10 minutes. Add water to a volume of 400 cc., in which all but possibly a small spongy mass of lead will be soluble. Let crystallize over night, decant and filter. Evaporate the filtrate to 100 cc. and again let crystallize. Filter and precipitate iron by rendering the filtrate faintly ammoniacal. Filter the iron and dissolve in 5 cc. of 1:3 hydrochloric acid. Transfer to a comparison tube and dilute to 20 cc. As a standard for duplication dilute 5 cc. of 1:3 hydrochloric acid with water to 20 cc.

*Zinc Oxide.* Iron in zinc oxide does not give the full color with thiocyanate. Dissolve 0.25–1.0 gram of zinc oxide in 5 cc. of concentrated hydrochloric acid. Extract the iron with ether in an all-glass modified Soxhlet apparatus<sup>19</sup> as illustrated.

Evaporate the ether from the extracted iron. Take up with 10 cc. of 1:1 hydrochloric acid and dilute to 45 cc. As standard use 10 cc. of 1:1 hydrochloric acid diluted to 45 cc. with distilled water. Compare by duplication.

Separation of zinc by precipitation with sodium ammonium phosphate in acetic acid solution has also been suggested.<sup>24</sup>

*Bismuth or Bismuth Oxide.* Dissolve a sample of 10–30 grams in 50 cc. of 1:1 hydrochloric acid. Dilute to 250 cc., filtering if necessary. Do not add nitric acid.<sup>19</sup> If bismuth in nitric acid contains iron a yellow color instead of red is produced with thiocyanate solutions. Take 10 cc. of sample and dilute to 95 cc. As standard add 2 cc. of 1:1 hydrochloric acid to distilled water and dilute to 95 cc. Compare by duplication.

An alternative method is to treat the bismuth or bismuth oxide in the same way as lead and its compounds and precipitate the bismuth from faintly acid solution as the sulfide.

*Battery acid.*<sup>6</sup> To a 10 cc. sample diluted to about 30 cc. add potassium permanganate solution until a faint color persists. As an alternative add a few mg. of potassium persulfate. Evaporate to a small volume and drive off sulfur trioxide fumes until the volume remaining is approximately 1 cc. Dilute to 45 cc.

As standard for duplication mix 1 cc. of concentrated sulfuric acid



FIG. 78

Apparatus for  
Solvent Extraction  
of Iron from  
Aqueous Solution

with water and dilute to 45 cc. If necessary extract the color with ether or a mixture of ether and amyl alcohol. This method is more sensitive than the ferrocyanide or pyrimidone reactions for application to battery acid.

*Water.* Evaporate 100 cc. or less to dryness.<sup>32</sup> If the sample contains much organic matter, ignite carefully. Let cool and moisten with 5 cc. of 1:1 hydrochloric acid. Warm 2 or 3 minutes. Add 5 to 10 cc. of hot water, warm a few minutes longer and rinse into a comparison tube, filtering if necessary. Add a few drops of approximately 0.2 *N* potassium permanganate solution until the color persists at least 5 minutes. Let cool and dilute to 95 cc. Compare with standards containing 5 cc. of 1:1 hydrochloric acid and distilled water to make a volume of 95 cc., allowing for the volume of standard iron solution to be used.

*Water, Alternative Method.*<sup>32</sup> If low in organic matter, boil 50 cc. of the sample with 5 cc. of 6 *N* nitric acid for 5 minutes, add 3 drops of 0.2 *N* potassium permanganate solution, cool and dilute to 95 cc. Compare with standards containing 5 cc. of 6 *N* nitric acid to the same final volume of 100 cc.

*Water, Alternative Method.*<sup>32</sup> For surface waters low in organic matter iron may first be separated. Evaporate a suitable portion with 2-3 cc. of concentrated nitric acid to about 50 cc. Add permanganate, if necessary, to destroy organic matter. Add ammonium hydroxide to the hot solution and continue heating until the odor of ammonia is hardly discernible. Filter and wash with water containing a little ammonia, at 70-80°. Dissolve the precipitate in 5 cc. of concentrated hydrochloric acid and transfer to a comparison tube. Cool and dilute to 45 cc.

Standards for comparison should contain 5 cc. of concentrated hydrochloric acid in a total volume, after addition of reagent, of 50 cc.

Other modifications<sup>35-38</sup> have been published.

*Water, Ferric Iron.*<sup>39</sup> If the iron content is sufficiently high ferric iron can be estimated directly in water. Siphon 90 cc. into a tube filled with carbon dioxide gas so that air cannot oxidize the iron. Add 5 cc. of 1:4 hydrochloric acid. As standard take 90 cc of distilled water and 5 cc. of 1:4 hydrochloric acid. If the color of the sample is not sufficiently

<sup>32</sup> American Public Health Association, "Standard Methods of Water Analysis," 5th ed., p. 47 (1923).

<sup>35</sup> P. Lehmann and A. Renss, *Z. Untersuch. Lebensm.* 54, 374-6 (1927).

<sup>36</sup> L. A. van de Vlugt, *Chem. Weekblad* 25, 495-6 (1928).

<sup>37</sup> L. W. Winkler, *Pharm. Zentralhalle* 74, 129-32 (1933).

<sup>38</sup> R. Czerny, *Wasser* 6, 155-67 (1932).

<sup>39</sup> O. Mayer, *Z. Untersuch. Lebensm.* 60, 195-210 (1930).

intense, and it will not usually be, apply the extraction method to concentrate it.

*Water, Ferrous Iron.*<sup>39</sup> Subtract the estimated ferric iron from the total iron to obtain the value for ferrous iron.

*Water, Inorganically Combined Iron.*<sup>39</sup> Follow the procedure for ferric iron except that a trace of bromine is dissolved in the 1:4 hydrochloric acid used. Extraction of the resulting color with ether-amyl alcohol is usually necessary.

*Chalybeate Preparations.*<sup>42</sup> The methods for water are applicable to these iron preparations after suitable dilution.

*Sea Water.*<sup>43</sup> Collect the sample in well-seasoned bottles to avoid possible leaching of iron from the container. The analysis is desirably carried out while the water is fresh as the growth of diatoms may remove most of the iron. Filter waters rich in plankton immediately on receiving. If there is a delay before analyzing, acidify the water with sulfuric acid before storing.

Measure 100 cc. of water into a 500 cc. Erlenmeyer flask. Add 6 cc. of concentrated sulfuric acid and evaporate to sulfur trioxide fumes. This removes chlorides, fluorides, nitrates and nitrites and destroys the organic complexes in which the iron is present. While still warm, carefully add 85 cc. of water and heat the covered flask on a steam bath until alkaline earth sulfates are dissolved. Add 0.63 per cent potassium permanganate solution, drop by drop, until the color persists. Two drops are normally sufficient. Decolorize excess permanganate with 1 cc. of bromine water and boil off excess bromine. The solution should be clear, except possibly for a deposit of white silicious material. When the solution has cooled, transfer it to a 100 cc. Nessler tube and use for development of color.

Prepare a sodium sulfate solution for use in the standards by dissolving 450 grams of hydrated sodium sulfate in water to make 1 liter. This is approximately saturated at 20°. To this add 0.4 cc. of 50 per cent sodium hydroxide solution. Heat and stir to precipitate iron. Filter and neutralize with 1:1 sulfuric acid.

To 22 cc. of the sodium sulfate solution add 50 cc. of water and 5 cc. of concentrated sulfuric acid. This is equivalent to the sodium present in sea water in the form of various salts. As a series, add suitable amounts of iron in the range up to 0.20 mg. per liter and treat these in the same

<sup>42</sup> K. Scheringa, *Pharm. Weekblad* 68, 735-8 (1931).

<sup>43</sup> Thomas G. Thompson, Raymond W. Bremner and I. Marion Jamieson, *Ind. Eng. Chem., Anal. Ed.* 4, 288-90 (1932).

way as the sample. The color in the sample and standards will be more brownish than is normal for the iron determination.

*Milk.*<sup>44,45</sup> Evaporate 100 cc. of milk in platinum to dryness on a water bath. Transfer the dish to an electric muffle and carbonize slowly with the door open. Gradually close the door and leave it over night just below a dull red heat. There is no loss of iron or fusion of the ash. Take up the ash with 3 cc. of 1:1 hydrochloric acid and filter, reserving the filtrate. If the residue shows any carbon, ash as before and take up with 2 cc. of 1:1 hydrochloric acid. Filter as before and join the filtrates.

Add 10 cc. of concentrated hydrochloric acid and dilute to 50 cc. Boil for 20 minutes to hydrolyze phosphates. When cool, add 2 drops of concentrated nitric acid and dilute to 45 cc. Add sufficient 0.1 N potassium permanganate solution to give a faint pink color which persists for 20 seconds. As standard for duplication use 10 cc. of concentrated hydrochloric acid and 2 drops of concentrated nitric acid diluted to 44 cc. Pyrophosphate must be hydrolyzed to orthophosphate. The iron in milk is largely in the fat fraction. The method will detect 0.02 of iron.

An alternative treatment calls for boiling the solution of the ash rendered alkaline with sodium hydroxide solution, for 1 hour.<sup>46,47</sup> Both iron and copper can be extracted from the fat left by wet ashing.<sup>48</sup>

*Urine.* To 100 cc. of a 24-hour specimen of urine in a 300 cc. Kjeldahl flask add 10 cc. of concentrated sulfuric acid.<sup>49</sup> Boil until foaming starts. Heat cautiously until the frothing ceases and a homogeneous liquid results. Dilute, and transfer to a 50 cc. volumetric flask. When cool, to volume. Transfer appear discontinue

acid to metaphosphoric acid  
Add 2 cc. of water and transfer  
volumetric flask. Add 1 cc. of concentrated sulfuric acid and cc.

<sup>44</sup> H. Lachs and H. Friedenthal, *Biochem. Z.* 32, 130 (1911).

<sup>45</sup> Ralph Stugart, *Ind. Eng. Chem., Anal. Ed.* 3, 390-3 (1931).

<sup>46</sup> C. A. Elvehjem, *J. Biol. Chem.* 86, 463-7 (1930).

<sup>47</sup> Cf. C. A. Elvehjem and E. B. Hart, *J. Biol. Chem.* 67, 43-51 (1926).

<sup>48</sup> W. Williams, *J. Dairy Research* 3, 93-100 (1931).

<sup>49</sup> F. S. Fowweather, *Biochem. J.* 20, 93-8 (1926).

of acetone. Dilute to 20 cc. When cool compare by duplication. About 0.01 mg. of iron should be required.

As standard mix 1 cc. of concentrated sulfuric acid, 12.5 cc. of acetone and water to make 20 cc. If the sample after development of color is turbid, centrifuge and compare the color of the supernatant liquid. Some inorganic salt is usually precipitated by the acetone. The color fades fairly rapidly so that comparison should be made within a few minutes. While this procedure is more difficult than those for tissue or blood it is relatively simple as compared with others for urine.

*Blood by peroxide oxidation.* Measure 1 cc. of blood into a test tube containing 4 cc. of water.<sup>49</sup> Mix and transfer 1 cc. of diluted blood to a 25 × 200 mm. Pyrex test tube. Add 1 cc. of concentrated sulfuric acid. Boil until white fumes appear. Discontinue heating a half minute and add 0.5 cc. of 30 per cent hydrogen peroxide dropwise from a pipet. Boil again to the appearance of white fumes. Cool and add hydrogen peroxide as before. When again heated the solution should become colorless. Add 0.3 cc. of hydrogen peroxide and heat a minute after the solution has become colorless. Let cool, dilute with about 5 cc. of water and transfer to a comparison tube. In a similar tube put 1 cc. of concentrated sulfuric acid. Add water to about 18 cc., then 25 cc. of acetone. Mix, let cool and dilute to 45 cc. Compare by duplication. About 0.1 mg. of iron should be required. The standard may also be prepared with an addition of 0.1 mg. of iron and compared by balancing or dilution. Blood solids may also be ashed by fushion with potassium bisulfate.<sup>51</sup>

*Blood by perchloric acid oxidation.*<sup>52</sup> Measure 1 cc. of blood into a 100 cc. Kjeldahl flask. Add 5 cc. of concentrated sulfuric acid and 2 cc. of 60 per cent perchloric acid. Digest over a low flame. It will be colorless in about 10 minutes. Heat to sulfur trioxide fumes and cool. Add a drop of concentrated nitric acid and dilute to 100 cc. Pipet 20 cc. into a comparison tube.

As standard take 1 cc. of concentrated sulfuric acid. Add 0.4 cc. of 60 per cent perchloric acid and 1 drop of nitric acid. Dilute to 20 cc. If necessary for greater sensitivity extract the color developed with amyl alcohol before comparing.

*Blood without ashing.*<sup>53</sup> To 0.5 cc. of blood in a 50 cc. volumetric flask add 2 cc. of concentrated sulfuric acid and mix well. Add 2 cc. of

<sup>51</sup> W. Autenrieth and J. Koenigsberger, *Münch. med. Wochschr.* 57, 998-1001 (1910).

<sup>52</sup> R. P. Kennedy, *J. Biol. Chem.* 74, 385-91 (1927).

<sup>53</sup> Sin Yin Wong, *J. Biol. Chem.* 77, 409-12 (1928).

saturated potassium persulfate solution and shake. Add water to about 25 cc., follow with 2 cc. of 10 per cent sodium tungstate solution and mix. Cool, dilute to volume and mix well. Filter through a dry filter of filtrate as sample.

0.8 cc. of concentrated sulfuric acid, 0.8 cc. of

tungstate solution to

cc. Compare by di

containing 0.1 mg.

About 1 cc. of a standard

required. Results by this

are somewhat too high on pure hemoglobin

blood. Its reliability is therefore ques-

tioned.<sup>54</sup>

*Blood, Micro Method.* A micro method requires a very small quantity of blood and can be carried out in 10 to 15 minutes. Add 0.040 cc. of blood to 2 cc. of water, introduce 0.2 cc. of 0.1 *N* hydrochloric acid and 2 cc. of potassium dichromate solution.<sup>55</sup> Place in a water bath for 2 minutes. A brown-red coagulum appears over a slightly yellowish background. Add 1 cc. of concentrated hydrobromic acid to split off the iron. Boil for 5 minutes. A water-clear liquid containing a

residue, with ac-

etic acid and

0.1

acid with water to make 5 cc. Dilute

by duplication or add a

known amount of iron to compare by balancing. If the iron content is

less than 0.018 g. will be required for a 0.04 sample of blood. Citrate

salts used as preservatives do not interfere with this method.

An accuracy of 1 per cent may be expected.

*Plasma.*<sup>56</sup> Take 10–12 cc. of blood by venipuncture, being sure that the needle fits well so that air will not leak in and bubble through the

syringe. Expel the blood into a conical centrifuge tube which has been coated with wax, leaving about 2 cc. in the syringe as rejected. Centrifuge

10 minutes to separate 4–5 cc. of plasma. Transfer 2.5 cc. to a test

tube and about 2 cc. to another test tube. Test the 2 cc. portion for hemoglobin by benzidine and if the test is positive reject the sample.

To the hemoglobin-free plasma in a Pyrex test tube add 1.5 cc. of

<sup>54</sup> Tibor Laszlo, *Biochem. Z.* 237, 483-96 (1931).

<sup>55</sup> Louis Berman, *J. Biol. Chem.* 35, 231 (1918).

<sup>56</sup> F. S. Fowweather, *Biochem. J.* 28, 1160-4 (1934).



concentrated sulfuric acid, 0.5 cc. of 30 per cent hydrogen peroxide and a small piece of platinum wire to prevent bumping. Heat to boiling over a micro burner and drive off the water. Continue careful heating until decomposition is complete and sulfuric acid is distilling on the walls of the tube. If carbon remains, cool, add 0.5 cc. of 30 per cent hydrogen peroxide and again heat. When the liquid is colorless continue to heat until all hydrogen peroxide is decomposed. When cool dilute with 2 cc. of water, transfer to a 10 cc. volumetric flask and dilute to volume. As standard in another flask take 1 cc. of concentrated sulfuric acid and dilute to volume with water. To each of these add 10 cc. of acetone and use as sample and standard for development of color by duplication.

*Tissue.* Mince the tissue or organs and wash with water to remove blood.<sup>49</sup> Dry and grind in a mortar until the ground sample passes through a 30-mesh sieve. Weigh 1 gram, or less if the organ contains more than a normal amount of iron, into a 300 cc. Kjeldahl flask. Add 10 cc. of concentrated sulfuric acid. Heat gently for 15 minutes and then strongly for 30 minutes. Let cool, dilute with an equal volume of water, and transfer to a 50 cc. stoppered volumetric flask. When completely cool dilute to 50 cc. A flocculent precipitate is formed. Mix well and transfer 5 cc. at once to a Pyrex tube. Add 1 cc. of 30 per cent hydrogen peroxide and heat to boiling. The solution should become colorless or faintly yellow. Further additions of peroxide as under "Blood"<sup>57</sup> may be necessary to destroy all organic matter. Let cool and transfer to a comparison tube.

As standard add to a similar tube 1 cc. of concentrated sulfuric acid. Dilute sample and standard to about 18 cc. To each add 25 cc. of acetone and mix. Dilute to 45 cc. Compare by duplication. The error found was 1 to 3 per cent.

*Tissue. Alternative Method.*<sup>52</sup> Pieces of tissue up to about 3 grams can be digested by the procedure outlined for blood with perchloric and sulfuric acids.<sup>57</sup> No drying or grinding is necessary.

*Tissue by Dry Ashing.*<sup>45</sup> Use the method for milk by dry ashing, suitably modified as to sample.

*Feces.*<sup>61</sup> Digest a suitable sample with concentrated sulfuric acid and 30 per cent hydrogen peroxide as described under the first method for blood.<sup>57</sup>

<sup>57</sup> See p. 291.

<sup>61</sup> Giorgio Dominici, *Bull. soc. ital. biol. sper.* 3, 1047-9 (1928).

*Wine, Total Iron.*<sup>63</sup> Evaporate 25 cc of wine to dryness and carefully ash without heating to redness. Dissolve in 5 cc. of 1:1 hydrochloric acid and dilute to 45 cc. In a test tube mix 5 cc. of 1:1 hydrochloric acid with 40 cc. of water. Compare by duplication.

The iron may also be oxidized by 3 drops of 3 per cent hydrogen peroxide per 5 cc. of wine. This is unnecessary.<sup>64,65</sup>

*Wine, Ferric Iron.* This method is only to white wine and with iron is extracted

for total iron.

*Tanning Extracts.*<sup>69,70</sup> Select a sample to contain at least 0.05 mg. of iron. Details of the quantities of various liquors required for the separation of the iron are given under "Copper."<sup>71</sup> Dissolve the ferric nitrate of iron and aluminum hydroxides in 5 cc. of hot 1:1 hydrochloric acid and dilute to 45 cc. with water. As standard for duplication use 5 cc. of 1

per cent

has been ignited at too

d F

out to avoid i

1-2 grams of dried

mains, let

ric acid a

edn

add 2 cc. of concentrated

<sup>63</sup> A. Hanak, *Z. Untersuch. Lebensm.* 60, 291-7 (1930).

<sup>64</sup> L. Ferre and A. Michel, *Ann. fals.* 26, 18-26 (1933).

<sup>65</sup> J. Ribereau-Gayon, *Ann. fals.* 26, 297-9 (1933).

<sup>67</sup> J. Ribereau-Gayon, *Ann. fals.* 26, 224-33 (1933).

<sup>69</sup> L. Vangasbecq, *Ann. chim. anal. chim. appl.* 12, 168 (1930).

<sup>70</sup> M. P. Balfe and H. Phillips, *J. Intern. Soc. Leather Trades Chem.* 15, 226-35 (1931).

<sup>71</sup> See p. 151.

<sup>71a</sup> Harley A. Daniel and Horace J. Harper, *J. Assoc. Official Agr. Chem.* 17, 286-9 (1934).

containing 0.05–0.1 mg. of iron. Add 10 cc. of 3 *N* nitric acid to this and evaporate to dryness. Add another 10 cc. of 3 *N* nitric acid and again evaporate to dryness. Add 20 cc. of 0.5 *N* nitric acid and heat for 10 minutes. Add 70 cc. of water and use as sample.

*Plants.*<sup>72</sup> Follow the preparation of sample given for aluminum<sup>73</sup> up to separation of aluminum and iron. Decant the aluminum solution completely from the iron precipitate. Dissolve the precipitate in 5 cc. of hot 1:1 hydrochloric acid and dilute to 45 cc. with water. As a parallel standard for duplication, dilute 5 cc. of 1:1 hydrochloric acid to 44 cc. with distilled water.

*Textiles.*<sup>74</sup> Follow the preparation of sample for copper<sup>75</sup> until rendered just acid to Congo red. Add 5 cc. of 10 per cent sulfuric acid, and 0.1 *N* permanganate to a faint pink color. Dilute to 95 cc. As a standard for duplication, add 2.5 grams of potassium sulfate, 2.5 grams of sodium sulfate and 10 grams of ammonium sulfate to water. Dissolve, add 5 cc. of 10 per cent sulfuric acid and dilute to 94 cc.

**Thiocyanic Acid Reagent**—Iron present in thiocyanates can be removed by conversion of the thiocyanates to thiocyanic acid and subsequent extraction of the latter. By making the thiocyanate solution strongly acid the iron is not extracted.<sup>76</sup>

As a suitable method of purification<sup>77</sup> dissolve 100 grams of ammonium thiocyanate, which need not be iron-free, in a cooled mixture of 65 cc. of concentrated sulfuric acid and 100 cc. of water. The temperature falls. Extract in a separatory funnel with about three-fourths the volume of amyl alcohol. Iron remains in the acid layer and the amyl alcohol layer is colorless. More acid will cause some decomposition of thiocyanic acid. If more amyl alcohol is used some iron will be extracted. The amyl alcohol layer contains 20 to 21 per cent of thiocyanic acid as shown by silver nitrate titration. On standing it soon becomes yellow and after sufficient time isoperthiocyanic acid is produced.

Extract the amyl alcohol layer at once with an equal volume of distilled water. This will contain about 10 per cent of thiocyanic acid, indi-

<sup>72</sup> I. B. Winter, *J. Assoc. Official Agr. Chem.* 13, 220-3 (1930).

<sup>73</sup> See pp. 263-4.

<sup>74</sup> Wm. C. Smith, *Proc. Am. Assoc. Textile Chem. Colorists*, 1930, 217-9; *Am. Dyestuff Repr.* 19, 583-5 (1930).

<sup>75</sup> See p. 155.

<sup>76</sup> H. N. Stokes and J. R. Cain, *J. Am. Chem. Soc.* 29, 443-7 (1907).

<sup>77</sup> John H. Yoe, "Photometric Chemical Analysis," 1st ed., vol. I, p. 221. John Wiley and Sons, New York (1928).

cating that the coefficient of partition is about unity. Repeat the extraction. Combine the aqueous extracts to give an aqueous solution containing 7.5 per cent of thiocyanic acid. The amyl alcohol layer may also be further extracted to give weaker aqueous extracts.

Saturate the aqueous thiocyanic acid with mercuric thiocyanate, leaving an excess of the latter in the bottle. By addition of a trace of potassium persulfate, no isothiocyanic acid will form for hours. A trace of iron from the mercury salt is permissible since an equal amount will be added to sample and standard.

Commercial mercuric thiocyanate is usually unreliable so that it is preferable to prepare the reagent by the following procedure.<sup>78</sup> Dissolve 27 grams of mercuric chloride in 75 cc. of hot water. Pour while hot into a hot solution of 7.6 grams of ammonium thiocyanate in 25 cc. of water. On cooling, mercuric thiocyanate crystallizes in needles. Filter and wash with cold water. Not all the excess chloride is removed but it does not interfere.

As an alternative method of purification, render the solution of potassium or ammonium thiocyanate distinctly acid with sulfuric acid and extract iron with about  $\frac{1}{2}$  volume of a 5:2 mixture of amyl alcohol and ether.<sup>79</sup> The iron free solution of reagent can then be used.

**Procedure**—The preparation of sample has been so outlined that, except in special cases noted, addition of 5 cc. of thiocyanate solution will give the color. The intensity of color can be varied to a considerable extent by the concentration of thiocyanate used. A 20 per cent solution of pure ammonium thiocyanate is often used and is specified in the procedures which follow. Alternatively a 7.5 per cent thiocyanic acid solution purified as above may be added.

If the color developed is too intense repetition with a weaker thiocyanate or thiocyanic acid solution will give a less intense color. The same change must be made with the standard. If both sample and standard are too dark both may be diluted to the same extent for easier comparison. While ammonium thiocyanate is specified throughout there is no reason why the corresponding sodium or potassium salts cannot be used.

**Duplication.** This is the oldest<sup>80,81</sup> and most reliable method. To standard and sample, of equal volume and acidity, add 5 cc. of 20 per cent

<sup>78</sup> John H. Yoe, *loc. cit.* p. 220.

<sup>79</sup> H. L. Smith and J. H. Cooke, *Analyst* 51, 503-10 (1926).

<sup>80</sup> Thornton J. Herapath, *J. Chem. Soc.* 5, 27 (1853).

<sup>81</sup> A. Thompson, *Chem. News* 51, 259 (1885).

ammonium thiocyanate. The color of the sample develops at once. Add to the standard a known solution of iron salt until the color matches that of the sample. If the color developed by the sample is too dark for duplication, dilute the sample and standard with distilled water, to the same extent.

If any question arises as to the sample and standard remaining oxidized add a few mg. of potassium persulfate<sup>82</sup> or 1 cc. of 30 per cent hydrogen peroxide to each tube.<sup>83</sup> When sample and standard are of the same color carefully adjust the volumes with distilled water and if necessary add more standard to bring the colors to a match.

*Balancing Method.* This method is reliable only within a small range of variation between sample and standard. To a suitable blank containing the same materials as the sample add a suitable amount of standard iron solution, dilute to the same volume as the sample and add 5 cc. of 20 per cent ammonium thiocyanate solution. Add the same amount of reagent to the sample. Compare in one of the usual types of comparator.

*Dilution Method.* This, like the balancing method, can be used only if the variation between sample and standard is small. To both sample and blank plus standard in equal volumes add 5 cc. of 20 per cent ammonium thiocyanate solution and cautiously dilute the darker solution to obtain a match.

*Ether-Amyl Alcohol Method.*<sup>84,22</sup> If the color developed is not dark enough to read satisfactorily take 10 cc. or 25 cc. of the treated portions of each, sample and standard, in comparison tubes. To each add 5 cc. of a mixture of equal volumes of ether and amyl alcohol. Shake well and compare the colors of the upper layers. This procedure is particularly applicable for amounts less than 5 p.p.m. of iron. The sample should be one to which no acetone has been added.

**Standard—From Ferrous Ammonium Sulfate.**<sup>24</sup> Dissolve 0.7022 gram of ferrous ammonium sulfate in 100 cc. of water and add 5 cc. of concentrated sulfuric acid. Warm and oxidize with approximately 0.2 N potassium permanganate solution until the iron solution remains faintly pink. Cool and dilute to 1 liter. One cc. corresponds to 0.1 mg. of ferric iron.

*From Ferric Alum.*<sup>22</sup> Dissolve 0.630 g. of ferric alum in water, add

<sup>82</sup> H. N. Stokes and J. R. Cain, *J. Am. Chem. Soc.* 29, 412 (1907).

<sup>83</sup> W. B. Walker, *Analyst* 50, 279-83 (1925).

<sup>84</sup> Launcelot Andrews, *Chem. News* 70, 165 (1894).

5 cc. of concentrated sulfuric acid and dilute to 1 liter. Each cc. corresponds to 0.005 mg. of ferric oxide or to 0.0035 mg. of iron.

*From Ferric Chloride.*<sup>88</sup> The crystallized salt,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , has been proposed as a standard. The use of 0.0436 gram per liter contains 0.009 mg. of iron per cc.

*From Iron Wire.*<sup>52</sup> Dissolve 0.1 gram of analytical-grade iron wire in 10 cc. of 10 per cent sulfuric acid. Add 3 cc. of concentrated nitric acid and dilute to 1 liter. Each cc. contains 0.1 mg. of iron.

#### IRON BY THIOLYGLICOLIC (MERCAPTOACETIC) ACID

When thioglycolic acid is added to a solution containing ferric iron a blue to purple coloration of ferric thioglycolate first results.<sup>90-92</sup> This may be reduced by the reagent to the ferrous form, resulting in a red to purple color. The blue<sup>93</sup> or the pink<sup>94</sup> color is applicable to quantitative estimation. The blue to purple color is preferable for this purpose.<sup>94a</sup>

The color in alkaline solution tends to fade on standing but is readily brought back by shaking with air. The color follows Beer's law. It is well adapted to iron in whole blood,<sup>95</sup> giving somewhat higher results than the thiocyanate method.

The presence of 0.1 per cent of common positive and negative ions shows no effect. The interference of substances which themselves produce a color with the reagent may often be destroyed by the addition of sodium citrate. As much as 500-750 mg. of ortho- or pyrophosphoric acid per 0.1 mg. of iron does not interfere.<sup>96</sup> With small amounts of iron, fading does not occur for 30 minutes.<sup>97,98</sup> The accuracy is probably within 2 per cent.

**Sample—Milk.**<sup>93</sup> Digest 5 cc. of milk or 5 grams of dried milk with 3 cc. of concentrated sulfuric acid and 0.5 cc. of 60 per cent perchloric

<sup>88</sup> L. Berman, *J. Biol. Chem.* 35, 231-6 (1918).

<sup>90</sup> Rudolph Andreasch, *Ber.* 12, 1391 (1879).

<sup>91</sup> Peter Claesson, *Ber.* 14, 412 (1881).

<sup>92</sup> J. Ginsburg and S. Bondzynski, *Ber.* 19, 116 (1886).

<sup>93</sup> Gladys Leavell and N. R. Ellis, *Ind. Eng. Chem., Anal. Ed.* 6, 46-7 (1934).

<sup>94</sup> Edward Lyons, *J. Am. Chem. Soc.* 49, 1916 (1927).

<sup>94a</sup> Sidney L. Tompsett, *Biochem. J.* 28, 1536-43 (1934).

<sup>95</sup> Ben R. Burmester, *J. Biol. Chem.* 105, 189-98 (1934).

<sup>96</sup> R. F. Hanzel, *Proc. Soc. Exptl. Biol. Med.* 30, 846 (1933).

<sup>97</sup> L. Michaelis and E. A. Guzman Barron, *J. Biol. Chem.* 83, 191-210 (1929).

<sup>98</sup> Robert K. Cannon and George M. Richardson, *Biochem. J.* 23, 1242-62 (1929).

acid in a micro Kjeldahl flask. Heat for 10 minutes, let cool and add 0.5 cc. more of perchloric acid. Repeat until 2 cc. of the perchloric acid have been added, if necessary. When the solution is colorless, transfer to a beaker and rinse the flask with 5 cc. of water. Cool and add concentrated ammonium hydroxide until alkaline to litmus. Then add concentrated sulfuric acid until just acid and 10 drops in excess.

*Blood. Micro Method.* Digest a 0.01 or 0.02 cc. sample with 1 cc. of concentrated sulfuric acid and 1 cc. of 60 per cent perchloric acid. Then carry on the rest of the treatment as for milk.

*Blood.<sup>100</sup> Alternative Method.* Transfer 0.2 cc. of blood to a tube calibrated at 10, 15 and 20 cc. Add 1 cc. of 1:1 sulfuric acid and a bead to prevent bumping. Heat until water is driven off. As soon as the sample chars, let it cool for a minute and add 4 drops of 30 per cent hydrogen peroxide, drop by drop. Heat until clear and for 5 minutes longer. Let cool, and dilute to 10 cc.

*Urine or Feces.* Evaporate the sample to dryness and ash in an electric furnace. Dissolve the ash in 5 cc. of 1:2 hydrochloric acid. Filter to remove insoluble residue and wash the filter until free from acid. Dilute the filtrate and washings to a suitable volume, such as 50 cc. or 100 cc.

Transfer an aliquot, such as 25 cc., to a tube. Add 0.1 *N* potassium permanganate solution until a permanent pink color is obtained. This oxidizes all of the iron to the ferric condition. Add 5 drops of a freshly prepared 9 per cent solution of cupferron. Mix and centrifuge thoroughly for 4 minutes. This throws down all of the iron free from any possible interfering substances.

Decant and wash the precipitate twice with 1 cc. portions of water. Add 1 cc. of 1:1 sulfuric acid to the iron residue and heat. When the precipitate is well charred, let cool and add 30 per cent hydrogen peroxide, drop by drop, with intermittent heating until a clear solution is obtained. Let the tube cool and dilute with water to 10 cc.

**Procedure**—Prepare the reagent by adding 4 cc. of thioglycolic acid to 8 cc. of concentrated ammonium hydroxide in 50 cc. of water.

Add 1 cc. of the reagent to the sample and to a series of suitable standards diluted to about the same volume. Add concentrated ammonium hydroxide until a good purple color is developed, mix and compare. This is at pH 8.0 to 10.0. If the sample or standards fade before comparison, shake vigorously with air. The standards can often be brought back to color, even if 24 hours old.

<sup>100</sup> R. F. Hanzel, *Proc. Soc. Exptl. Biol. Med.* 30, 846 (1933).

## FERROUS AND FERRIC IRON BY THIOSALICYLIC ACID

Iron forms a yellow compound with thiosalicylic acid in ammoniacal solution.<sup>101</sup> The intensity of the color is independent of the concentration of ammonium hydroxide within fairly wide limits. In acid solution the same reagent gives a red compound with iron, but the depth of color is influenced by the concentration of acid as well as by that of the iron. The presence of oxidizing agents causes the yellow color to turn brown. Copper, nickel and cobalt, if present in large amounts, produce a green or brown color. The green color from copper may be removed by the addition of a few drops of a potassium cyanide solution.

Since the color in acid solution is only that of the ferric iron, and the color in alkaline solution shows all of the iron, the method is applicable to determination of both forms.<sup>102,103</sup> It is suitable for micro determinations, for example, using 0.1 cc. of blood, but before a determination is made the iron must be in the inorganic form. Accuracy is to within 1 to 4 per cent.

**Procedure—Ferric Iron.**—Dilute a portion of sample containing not more than 2 mg. of iron to about 20 cc. Add 10 cc. of 5 per cent ammonium chloride solution and 1 cc. of a cold saturated solution of  $\alpha$ -dinitrophenol. If the solution is alkaline to this indicator, add 0.1 *N* hydrochloric acid until the yellow color disappears. Carefully add 0.1 *N* sodium hydroxide solution until a permanent yellow color is obtained.

Dissolve 10 grams of thiosalicylic acid in 20 cc. of water. Add 10 per cent sodium hydroxide solution until pH 2 is reached and dilute to 100 cc. Prepare a citrate buffer as follows: Dissolve 21 grams of citric acid in 200 cc. of *N* sodium hydroxide solution. Dilute this to 1 liter. Mix 3 cc. of this with 7 cc. of 0.1 *N* hydrochloric acid for use. To the sample add 5 cc. of the thiosalicylic acid reagent and 5 cc. of the citrate buffer solution. Mix well and compare after 4 hours with standards similarly treated.

**Total Iron.** To the same amount of sample as for ferric iron, similarly diluted, add 10 cc. of *N* hydrochloric acid, 2 cc. of 20 per cent thiosalicylic acid and 20 cc. of the sodium citrate solution to which no acid has been added. Add 10 per cent sodium hydroxide solution until the color is changed from red to yellow.

Prepare a borate buffer by solution of 12.40 grams of boric acid in 100 cc. of *N* sodium hydroxide solution. Dilute to 1 liter and mix 46 cc.

<sup>101</sup> L. Lorber, *Biochem. Z.* **181**, 391 (1927).

<sup>102</sup> L. N. Lapin and W. E. Kill, *Z. Hyg. Infektionskrankh.* **112**, 719-23 (1931).

<sup>103</sup> F. Alten, W. Weiland and E. Hille, *Z. anorg. allgem. Chem.* **215**, 81-9 (1933).



of this with 54 cc. of *N* sodium hydroxide solution. To the sample solution add 10 cc. of *N* sodium hydroxide solution and 20 cc. of the borate buffer solution. Mix and compare after 4 hours with standards similarly treated.

*Ferrous Iron.* Subtract the value for ferric iron from that for total iron.

### IRON BY SALICYLIC ACID

Ferric iron in reaction with salicylic acid produces an amethyst color, ferrous iron produces no color. The color is destroyed by hydrochloric, nitric, or sulfuric acid. Phosphates, thiosulfates, sulfites, bisulfites and fluorides interfere. The color is stable for 48 hours in the presence of acetic acid. It fades fairly rapidly in sunlight. The method has been applied in particular to copper alloys<sup>104</sup> and to zinc.<sup>105</sup> The iron present should not exceed 1 mg. per 100 cc. of final solution. Accurate comparison of less than 0.01 mg. is impossible. The method is reported as sensitive to 0.4 p.p.m.

**Sample—Metals and Alloys.**<sup>105</sup> Dissolve a 2 gram sample in 20 cc. of concentrated hydrochloric acid. Add a few drops of concentrated nitric acid to oxidize the iron. Add 10 cc. of a solution containing 0.0211 gram of uranyl nitrate, which is equivalent to 10 mg. of uranium, then a slight excess of concentrated ammonium hydroxide. Heat to boiling and let stand 20 minutes. Filter, dissolve the precipitate in 10 cc. of 1:1 hydrochloric acid, and transfer to a comparison tube.

*Soil Extracts.*<sup>107</sup> Use the hydrochloric acid extract, taking an aliquot equivalent to about 0.1 mg. of iron. Dilute to about 10 cc. with 1:1 hydrochloric acid. For citric acid extracts, evaporate to dryness, burn off the citric acid and dissolve in 10 cc. of 1:1 hydrochloric acid. Transfer to a comparison tube.

**Procedure**—Add 1:1 ammonium hydroxide until the solution is nearly neutral, then 1 cc. of a 10 per cent sodium salicylate solution. Add a slight excess of 1:1 ammonium hydroxide. Add 1:1 acetic acid until the solution is just acid, when the color changes to purple. Add 10 cc. excess of 1:1 acetic acid and dilute to 100 cc.

<sup>104</sup> A. W. Gregory, *J. Chem. Soc. Trans.* 93, 93-5 (1908).

<sup>105</sup> W. J. Agnew, *Analyst* 53, 30 (1928).

<sup>107</sup> J. L. Steenkamp, *J. South African Chem. Inst.* 13, 64-70 (1930).

Prepare a standard tube with the same amount of uranyl nitrate solution, if used with the sample, and of acetic acid. Dilute to 95 cc. and add a ferric solution equivalent to 0.1 mg. of iron per cc.<sup>107a</sup> until the color matches that of the sample. Alternatively and preferably add a definite amount of standard to the blank and compare by balancing or dilution.

#### IRON BY SALICYLSULFONIC ACID

Micro estimation of iron in sputum has been carried out by the use of this reagent.<sup>108</sup>

**Procedure**—Ash with due precautions to avoid loss. Dissolve the ash in 2 cc. of 1:3 hydrochloric acid. Add 2 drops of 30 per cent hydrogen peroxide and 0.5 cc. of a 10 per cent solution of salicylsulfonic acid. A pink color develops at once. Add 1:1 ammonium hydroxide until alkaline, when the color will change to yellow. Dilute to 5 cc.

Mix a suitable volume of standard iron solution with 2 cc. of 1:3 hydrochloric acid. Carry it through the remaining stages of treatment which the sample received and compare by balancing.

#### FERRIC IRON BY 7-iodo-8-HYDROXYQUINOLINE-5-SULFONIC ACID

An aqueous solution of 7-iodo-8-hydroxyquinoline-5-sulfonic acid is yellow. The color is altered by ferric ion to a green suitable for comparison with a series of standards.<sup>109</sup> The solution should be acid to methyl orange. Ferrous ion gives no color.

A slight greenish yellow distinguishable from the blank is given by 1 part in 15 million. A distinct greenish yellow is given by 1 part in 10 million, a bright bluish-green by 1 part in 1.5 million and green by increased concentrations.

The color developed is very stable to light. It is destroyed by strong acids or bases. Salts that hydrolyze easily should therefore not be present in substantial amounts. Copper gives a white precipitate which will interfere if more than a few parts per million are present. Colored ions not exceeding the amount of iron do not interfere.

This reagent has probably been reported in more detail as to possible interference by other ions than any other one. There is no color developed

<sup>107a</sup> See p. 297.

<sup>108</sup> J. Paviot, R. Chevalier and L. Revol, *Compt. rend. soc. biol.* 99, 1749-50 (1928); *Physiol. Abs.* 14, 142 (1930).

<sup>109</sup> John H. Yoe, *J. Am. Chem. Soc.* 54, 4139-43 (1932).

by lithium, sodium, potassium, rubidium, caesium, cupric, silver, auric, calcium, barium, strontium, beryllium, magnesium, zinc, cadmium, mercurous, mercuric, borate, aluminum, scandium, yttrium, lanthanum, cerium, praseodymium, neodymium, samarium, gadolinium, dysprosium, erbium, thulium, ytterbium, gallium, indium, thallium, titanite, zirconium, hafnium, thorium, germanium, stannous, stannic, lead, vanadium, columbium, tantalum, phosphate, arsenic, antimony, bismuth, chromic, molybdate, tungsten, uranyl, sulfide, selenate, tellurate, manganous, fluoride, chloride, bromide, iodide, ferrous, cobalt, nickel, ruthenium, rhodium, palladium, iridium or platinic ions.

**Procedure**—Be sure that the sample solution is only faintly acid, by addition of ammonium hydroxide if necessary. Transfer a suitable volume to a 50 cc. Nessler tube. Add 5 drops of a 2 per cent aqueous solution of the reagent. Mix and dilute to 50 cc.

Compare with a series of standards similarly prepared. These do not fade on standing.

#### IRON BY ACETYLACETONE

The color of a solution of ferric iron and acetylacetone is red by transmitted light and orange-red by reflected light, if fairly concentrated. This is due to replacement of one of the hydrogen atoms attached to the middle carbon atom in acetylacetone,  $\text{CH}_3\text{COCH}_2\text{COCH}_3$  by iron.<sup>110</sup> More dilute solutions are orange-red by transmitted light and yellow by reflected light.<sup>111</sup> This color lasts for at least 3 weeks. The method may be used to determine from 0.003 mg. to 0.6 mg. of iron per 50 cc. of solution. Few common inorganic salts that can exist in solution with iron have any influence on the color, if present in small amounts. The reagent can be used whenever the thiocyanate reagent can be used. Sodium and potassium hydroxides precipitate iron from a solution of ferric acetylacetonate. Ammonium hydroxide produces a yellow color but does not interfere with the determination. Oxides of nitrogen must be absent.

Small temperature changes do not show a definite effect on the color. When heated to boiling it is altered but returns to the original color on cooling.

The color does not vary uniformly with variation in depth of liquid so that only the duplication method is suitable. In aqueous solution the

<sup>110</sup> A. Combes, *Compt. rend.* 105, 868 (1887); *J. Am. Chem. Soc.* 54, 128 (1888).

<sup>111</sup> H. B. Pulsifer, *J. Am. Chem. Soc.* 26, 967 (1904).

compound of acetylacetone and iron is only slightly ionized but hydrolyzes readily to ferric hydroxide.<sup>112</sup> It is therefore necessary to standardize the concentrations of acid and reagent. The solubility of the compound is of the order of 1.5 grams per liter.<sup>111</sup>

**Procedure**—Evaporate the solution to dryness on the water bath. Add 5 drops of a mixture of equal volumes of concentrated sulfuric and nitric acids to the residue to destroy any organic matter present. Drive off excess acid by heating. Dissolve the residue in 10 cc. of distilled water. This solution of iron should contain no more than a drop of any strong acid. Transfer the solution to a comparison tube. Add 2 cc. of a 0.5 per cent aqueous or alcoholic solution of freshly distilled acetylacetone and dilute to 50 cc. Compare by duplication with a standard of 45 cc. of distilled water and 2 cc. of the same 0.5 per cent acetylacetone solution.

#### IRON BY POTASSIUM FERROCYANIDE

The blue color of ferric ferrocyanide may be used for the determination of iron.<sup>114,115</sup> The method is applicable in the presence of phosphoric acid but large amounts of copper interfere. Nitrous acid must be absent. This method may be used to determine the ferric iron in the presence of ferrous iron or it may be used to determine the entire iron content. In the latter case the solution must first be oxidized with potassium permanganate or nitric acid to bring all iron to the ferric condition.

Accuracy to 1.5 per cent on quantities as low as 0.01 mg. may be expected if the work is carefully done. Good agreement with the thiocyanate method has been reported<sup>116</sup> except on wines, where some techniques give low results.<sup>117</sup> By a special technique the results are used to determine the amount of ferrocyanide needed to "fine" white wine.<sup>118</sup>

**Sample—Organic.** Ignite 1 gram or more of an organic sample in a platinum crucible below redness. In the presence of hydrofluoric acid or fluorides treat with concentrated sulfuric acid and evaporate to remove hydrofluoric acid. Extract the ash twice with 7 cc. of 5:2 hydrochloric

<sup>112</sup> A. Hantzsch and C. H. Desch, *Ann.* 323, 1 (1902); *J. Am. Chem. Soc.* 82, 708 (1902).

<sup>114</sup> W. B. Walker, *Analyst* 50, 279-83 (1925).

<sup>115</sup> F. Pavelka and Hermine Morth, *Mikrochemie* 13, 305-12 (1933).

<sup>116</sup> G. S. Fraps and J. F. Fudge, *J. Assoc. Official Agr. Chem.* 15, 307-10 (1932).

<sup>117</sup> L. Ferré and A. Michel, *Ann. fals.* 26, 18-26 (1933).

<sup>118</sup> A. Hanak, *Z. untersuch. Lebensm.* 59, 506-11 (1930).

acid and filter.<sup>119</sup> This acid mixture extracts iron more efficiently than nitric acid. Evaporate the acid solution to dryness. Dissolve the residue in 3 cc. of concentrated nitric acid, boil a minute or so to destroy any nitrous acid present, and transfer to a comparison tube. Dilute to a suitable volume.

*Blood.*<sup>120</sup> Pipet 0.2 cc. into a suitable tube. Add 1 cc. of concentrated sulfuric acid and heat to boiling for 3.5 minutes. When cool add 1 cc. of a saturated solution of potassium chlorate and heat to boiling for 3 minutes. When cool add 0.3 cc. of saturated potassium chlorate solution and boil 2 minutes. When cool dilute to 10 cc. The sample may also be oxidized by boiling with a mixture of nitric and perchloric acids.<sup>121</sup>

**Procedure**—Add 1 cc. of a 1 per cent solution of potassium ferrocyanide to a 10 cc. aliquot of the sample and dilute to 20 cc. After 15 minutes compare with a standard solution of ferric iron treated simultaneously with the reagents specified. Prepare a series of standards with 0.05, 0.1, 0.2, 0.4, 0.6 and 0.8 cc. of standard iron solution containing 0.1 mg. of iron per cc.<sup>121a</sup> Each standard should contain 3 cc. of nitric acid and the same volume of reagent. If necessary for more accurate comparison, add sufficient iron to the solution just lighter in color than the sample to obtain a match.

The excess of potassium ferrocyanide introduces a yellow color which is confusing. This is largely obviated by observing the sample with artificial light transmitted through filter paper stained deep yellow with picric acid. The resulting effect is largely a matter of intensities of grey, the method being more photometric than colorimetric.<sup>122</sup> Gum ghatti or gum arabic solution can advantageously be added to render the suspension stable. Permanent standards prepared with methylene blue may also be used.<sup>121</sup>

#### FERROUS IRON BY POTASSIUM FERRICYANIDE

Ferrous iron may be determined by means of the deep blue color of a solution of ferrous ferricyanide.<sup>124</sup>

<sup>119</sup> W. R. Mummary, *Analyst* 51, 511 (1926).

<sup>120</sup> Frederick Reis and H. H. Chakmakjian, *J. Biol. Chem.* 92, 59-63 (1931).

<sup>121</sup> Andrée Drilhon and Marcel Drilhon, *Compt. rend. soc. biol.* 109, 1234-5 (1932).

<sup>121a</sup> See p. 297.

<sup>122</sup> Otto Folin and Haqvin Malmros, *J. Biol. Chem.* 83, 115-20 (1929).

<sup>124</sup> American Public Health Association, "Standard Methods of Water Analysis," 5th ed., p. 49 (1923).

**Procedure**—To 50 cc. of a clear solution of the sample add 10 cc. of 1 : 5 sulfuric acid, and 15 cc. of a 0.5 per cent solution of potassium ferri-cyanide, freshly prepared. Dilute to 100 cc. and compare with a standard solution of ferrous iron treated with the same reagents.

**Standard Ferrous Solution**—Dissolve 0.7022 gram of ferrous ammonium sulfate in freshly boiled distilled water, add 10 cc. of 1 : 5 sulfuric acid and dilute to 1 liter. Each cc. corresponds to 0.1 mg. of ferrous iron. This must be freshly prepared.

#### IRON AS THE SULFIDE

The determination of iron by the brown color of the sulfide in alkaline solution is particularly applicable to the examination of drinking water but may be adapted to any estimation of ferrous iron in the presence of ferric, or to the determination of the total iron content of a solution by reduction of ferric iron to the ferrous condition.<sup>125</sup> If the examination is to be made on water it is best made immediately after taking the sample. If it is not to be made at once add a few drops of 1 : 1 hydrochloric acid and 10 cc. of hydrogen sulfide solution to 100 cc. of sample and stopper the bottle tightly to prevent oxidation of ferrous to ferric iron. The sample should have an iron content between 0.3 and 1.5 mg. of ferrous iron per liter. If the amount exceeds this, the solution may be diluted. If the iron content is less than specified, use a larger volume of solution.

**Sample**—To determine the entire iron content, evaporate the solution to dryness with a small amount of hydrochloric acid and potassium chlorate. Dissolve the residue in warm water with the addition of 2 cc. of concentrated hydrochloric acid and 5 cc. of saturated hydrogen sulfide solution. After cooling filter this solution and dilute the filtrate to 100 cc. The opalescence of the filtrate disappears on the addition of the reagents. If the sample contains lead, treat with hydrochloric acid and hydrogen sulfide solution, let stand over night, then decant from the precipitate.

**Procedure**—Compare by the duplication method. For the sample use 100 cc. of the solution. To the sample and to 100 cc. of water add 5 cc. of an aqueous solution of hydrogen sulfide and 2 drops of concentrated ammonium hydroxide. Add the standard solution to the blank drop by drop until the colors of the 2 solutions are approximately equal.

<sup>125</sup> L. W. Winkler, *Z. anal. Chem.* **41**, 550 (1902).

The color of the standard will show more of a bluish hue than that shown by the sample and it is therefore necessary to decolorize the standard with a few drops of 1:1 hydrochloric acid and then renew the color by addition of a few drops of concentrated ammonium hydroxide. The color which comes back will be brown. Add more standard to the blank if necessary, in order to bring it to the point where the colors of the sample and standard are identical. Decolorize both sample and standard with a few drops of 1:1 hydrochloric acid and add a few drops of concentrated ammonium hydroxide. The two solutions should now match and should be equal in volume. If necessary, add distilled water to the sample.

**Standard**—Prepare a standard by dissolving 0.7022 gram of ferrous ammonium sulfate in water acidified with 10 per cent of sulfuric acid by volume, and saturated with hydrogen sulfide. Dilute with the same to 1 liter. This contains 0.1 mg. of ferrous iron per cc.

#### IRON AS THE CHLORIDE IN CONCENTRATED HYDROCHLORIC ACID

The yellow color of ferric chloride in concentrated hydrochloric acid has been adapted to the analysis of samples containing iron, when no interfering colors are present.<sup>126</sup> The amount of iron present must be less than 0.1 mg. per cc., and less than half of this concentration is better. The color intensity of ferric chloride reaches a maximum in 28 per cent hydrochloric acid. It also varies considerably with temperature.<sup>127</sup> The presence of free chlorine does not interfere but nitric acid or its oxides do. If copper is present it is removed by hydrogen sulfide, as copper chloride has the same color as iron. Manganese does not interfere. A small amount of cobalt or nickel will not produce sufficient color to obscure the results; larger amounts must be removed. Zinc and mercury tend to bleach the color. Calcium and magnesium chlorides intensify it. Sulfates decrease the color intensity. The method has been applied to blood ash.<sup>128</sup>

**Sample**—*Alloys or Metals*. Dissolve from 5 to 25 grams of alloy or metal in nitric acid and evaporate several times with concentrated hydrochloric acid until the brown nitric oxide fumes have disappeared. Take

<sup>126</sup> C. Hüttner, *Z. anorg. Chem.* **86**, 341-57 (1914).

<sup>127</sup> J. C. Hostetter, *J. Am. Chem. Soc.* **41**, 1531 (1919).

<sup>128</sup> G. Deniges, *Bull. soc. pharm. Bordeaux* **67**, 81-4 (1929).

up the residue with 1:1 hydrochloric acid. If no disturbing color is present iron may be determined directly. In the presence of nickel, separate the iron by precipitating it with ammonium hydroxide, redissolve in acid and reprecipitate. A large amount of nickel is carried down with the iron in the first precipitation. Dissolve the ferric hydroxide in approximately 20 per cent hydrochloric acid, made by a 1:1 dilution of the usual concentrated acid. As this is approximately the constant boiling ratio, the solution may be heated to boiling with little change in concentration. Dilute the sample to 50 cc. with 20 per cent hydrochloric acid.

*Salts and Oxides.* Iron is present in most commercial salts and oxides as scale, derived from the iron apparatus used in their manufacture. It is very resistant to the action of sulfuric and nitric acids. Dissolve iron scale by heating with 20 per cent hydrochloric acid for several hours. If the iron is not fully oxidized treat with an oxidizing agent, such as nitric acid or hydrogen peroxide. Add 1 cc. of 10 per cent aluminum sulfate solution, and precipitate the iron and aluminum with 1:1 ammonium hydroxide. Filter, wash and dissolve the residue in 1:1 hydrochloric acid. Dilute to 50 cc. with the same strength of acid.

*Solution.* If the iron is already in solution, evaporate a suitable sample just to dryness and take up with 1:1 hydrochloric acid. If nitric acid is present several evaporations to expel nitrous and nitric acid will be necessary. Dilute to 50 cc. with 1:1 hydrochloric acid.

**Procedure**—Prepare a series of standards from a ferric iron solution <sup>128a</sup> in 1:1 hydrochloric acid. Dilute sample and standards to the same volume with the 1:1 acid and compare. Comparison with a suitable standard by the dilution or balancing methods is also satisfactory. Sample and standard should have the same salt content. The sensitivity of the method may be greatly increased by adding the same amount of calcium chloride to each, to intensify the color. The maximum color is obtained in more concentrated hydrochloric acid but the 1:1 concentration is more convenient.

#### IRON BY DIMETHYLGLYOXIME

The red color produced by the reaction of ferrous iron with dimethylglyoxime is very distinctive and permits the estimation of the iron with an accuracy of 1 to 2 per cent.<sup>129</sup> The iron present should be between 0.01

<sup>128a</sup> See p. 297.

<sup>129</sup> L. Tschugaeff and B. Orelkin, *Z. anorg. Chem.* 89, 401-4 (1914).



and 0.06 mg. per cc. The presence of large amounts of aluminum and zinc must be avoided. Alkaline earths and magnesium do not interfere. The iron is obtained in the ferrous state by reduction with hydrazine.

**Procedure**—To 50 cc. of sample add 1 gram of hydrazine sulfate and 5 cc. of a saturated solution of dimethylglyoxime in 95 per cent alcohol. Heat to boiling. Add 10 cc. of concentrated ammonium hydroxide and boil for one-half minute. Cool rapidly and dilute to 100 cc. The comparison is carried out by any of the usual methods, using the standard iron solution prepared for preceding methods<sup>130</sup> and treated in the same way as the sample at the same time.

#### IRON BY PYROCATECHOL

Ferric iron gives a dark green color in faintly acid solution with pyrocatechol. In faintly alkaline solution, or if the concentration of iron is less than 0.01 *N*, the color is a brilliant violet,<sup>131</sup> suitable for colorimetric estimation.

**Procedure**—To 50 cc. of solution add 10 cc. of an aqueous 1 per cent solution of pyrocatechol. Shake and dilute to 100 cc. Compare with a fresh solution of ferric chloride, treated similarly.

**Standard**—Prepare a 0.002 *N* solution of ferric chloride. This contains 0.1081 gram of ferric chloride per liter. A sample of 50 cc. treated with the reagent and diluted to 100 cc. becomes 0.001 *N*. This corresponds to 0.0186 mg. of iron per cc.

#### IRON BY PYRAMIDONE

The reaction of iron with pyramidone to give a blue compound may be used in acid solution, the concentration of acid at 0.2 *N* or more having little influence on the color. As little as 0.05 mg. of iron per 100 cc. may be determined.<sup>132</sup>

**Procedure**—To a 50 cc. sample add 5 drops of concentrated sulfuric acid and 10 cc. of a 10 per cent solution of pyramidone in 0.2 *N* sulfuric

<sup>130</sup> See p. 297.

<sup>131</sup> A. L. Bernouilli, *Helv. chim. Acta* 9, 835 (1926).

<sup>132</sup> H. W. van Urk, *Pharm. Weekblad* 63, 1121-3 (1926).

acid. Dilute to 100 cc. with 0.2 *N* sulfuric acid. Compare with a standard iron solution <sup>132a</sup> similarly treated.

#### IRON BY ALLOXANTIN

Iron gives a blue color with alloxantin in alkaline solution.<sup>133</sup> The presence of citrates or tartrates causes no difficulty.

**Procedure**—Prepare the reagent by dissolving 0.1 gram of alloxantin in 10 cc. of 1.0 *N* sodium hydroxide solution. Destroy any color in the reagent by boiling, and cool rapidly. To 2 cc. of ferric iron solution add 1 cc. of reagent. Compare with a standard solution of ferric iron.<sup>134</sup>

#### IRON BY $\alpha, \alpha'$ -DIPYRIDYL

Ferrous iron reacts with 3 moles of  $\alpha, \alpha'$ -dipyridyl to form an intense red complex  $\text{Fe}[(\text{C}_{10}\text{H}_8\text{N}_2)_3]\text{X}_2$ , in which X is any monovalent acid radical.<sup>135</sup> The maximum color is obtained between pH 3.5 and 8.5.<sup>136</sup> The color is only influenced by other positive ions if their concentration greatly exceeds that of ferrous iron. Even then they may be overcome by use of sufficient reagent to insure transformation of the iron. Ferric iron has no more effect than other ions.

By estimation both before and after reduction, the method is applicable to estimation of ferrous and ferric iron. Sodium hyposulfite,  $\text{Na}_2\text{S}_2\text{O}_4$ , or hydrazine hydrate is suitable for reduction of ferric iron. The reagent does not react with reduced hematin or with pyridine-hemochromogen. It does not remove iron from hematin. Iodides, tungstates, alkaloidal reagents and thiocyanic acid interfere with development of the red color, usually by forming a precipitate. Manganese causes an interfering brown color.<sup>137</sup> Copper does not interfere up to 1000 times the amount of iron. Pyrophosphate does not interfere.

The reagent is ordinarily prepared by distillation of  $\alpha$ -picolinic acid although other methods are recorded. It is not as sensitive as some other known methods. The solubility is only 0.025 *M* at 20°. The method is accurate to 10 per cent by the series of standards method.

Results on egg yolk by this method check with determinations on ash.

<sup>132a</sup> See p. 297.

<sup>133</sup> G. Deniges, *Compt. rend.* 180, 519 (1925).

<sup>134</sup> See p. 297.

<sup>135</sup> Fritz Blau, *Ber.* 21, 1077 (1888); *Monatsh.* 19, 647 (1898).

<sup>136</sup> Robert Hill, *Proc. Roy. Soc. (London)* B107, 205-14 (1930).

<sup>137</sup> F. Feigl, P. Krumholz and H. Hamburg, *Z. anal. Chem.* 90, 199-202 (1932).

**Reagents—Dipyridyl.** Dissolve 5 grams of  $\alpha,\alpha'$ -dipyridyl in water and dilute to 1 liter.

**Sodium hyposulfite.** Dissolve sodium hyposulfite in water at 40° until a nearly saturated solution is obtained. Add the solution of reagent until the maximum color is developed. Filter on a Büchner funnel. Add an equal volume of alcohol to the filtrate to precipitate pure sodium hyposulfite. After 20 minutes filter and wash on the filter with 70 per cent alcohol until the crystals and washings are colorless. Wash with 95 per cent alcohol. Transfer the crystals to a beaker and boil with 95 per cent alcohol for 10 minutes. Filter at once and transfer the crystals to a desiccator over concentrated sulfuric acid. Prepare a 10 per cent solution of the colorless crystals in water.

**Acetate Buffer.** Mix 100 cc. of 2 per cent acetic acid solution and 100 cc. of 3 per cent sodium acetate solution.

**Sample—Nonhematin Iron in Yeast.** Wash 20 grams with distilled water until the filtrate gives no color with sodium hyposulfite and dipyridyl reagent. Suspend in 50 cc. of acetate buffer solution.

**Hematin Iron.**<sup>138</sup> Oxidize the organic matter with hydrogen peroxide and proceed as usual. If nonhematin iron is also present, it must be estimated separately and the value subtracted.

**Egg Yolk.** Boil the egg for 15 minutes. Separate and disintegrate the yolk. Suspend 20 grams in 50 cc. of acetate buffer solution.

**Grains, etc.**<sup>138a</sup> Wet-ash with concentrated sulfuric and nitric acids. Expel excess acid and dilute to about 0.1 *N* acidity. Add 25 cc. of acetate buffer solution.

**Procedure**—Add 5 cc. of dipyridyl reagent. Absence of a red color shows ferrous iron absent. Add 5 cc. of sodium hyposulfite reagent. Add 1 cc. of a saturated solution of sulfur dioxide in water. The red color which develops on addition of the hyposulfite diffuses out of the sample, if solid, into the solution after addition of the sulfur dioxide. After 2 hours filter and wash until the residue is colorless. Dilute to 100 cc. and compare with a series of standards similarly treated. Properly protected, these keep indefinitely.

#### IRON BY 8-HYDROXYQUINOLINE

The iron compound of 8-hydroxyquinoline has an intense dark green

<sup>138</sup> R. Hill and D. Keilin, *Proc. Roy. Soc. (London)* B114, 104-9 (1933).

<sup>138a</sup> D. Scharrer, *Z. Pflanzenernähr., Düngung Bodenk.* 33A, 336-40 (1934).

color in solution in organic solvents, which may be used for its colorimetric estimation.<sup>139</sup> While manganese, aluminum, copper and zinc also react they are present in relatively small amounts in biological materials and are not quantitatively precipitated at pH 5 under the conditions for precipitation of iron. The macro method given is accurate to about 0.01 mg. and the micro method to 0.001 mg. The method has been successfully applied to blood and milk.

**Sample—Biological material.** Ash a sample which will contain 0.05–0.5 mg. of iron or more, in porcelain. Platinum should not be used. Dissolve the ash by warming with 5 cc. of 1:1 hydrochloric acid and dilute to a known volume with water.

**Procedure—Macro.** Transfer a suitable aliquot of the sample solution to a 35–40 cc. centrifuge tube. Add 3 drops of glacial acetic acid, 3 drops of methyl red solution and 1 cc. of 2 per cent sodium oxalate solution. This will keep the iron in solution in the presence of phosphates and precipitate calcium. Add 5 per cent sodium hydroxide solution until the indicator begins to change color. Add 2 cc. of a 2.5 per cent solution of 8-hydroxyquinoline in glacial acetic acid and add 0.25 *N* sodium hydroxide solution drop by drop until the indicator changes color. Iron hydroxyquinolate separates. Heat the tube in a boiling water bath for 10 minutes. Centrifuge while warm to separate the iron hydroxyquinolate and calcium oxalate. Decant the upper layer and wash the precipitate with water. Again centrifuge and decant.

Dissolve the precipitate in 95 per cent alcohol to which a drop or two of 0.25 *N* sodium hydroxide solution has been added and dilute to 50 cc. or 100 cc. Compare with the color developed from a standard<sup>140</sup> iron solution similarly treated.

**Micro.** To 2 cc. of sample solution in a conical centrifuge tube add 1 drop of 25 per cent acetic acid, 1 drop of methyl red indicator solution and a few drops of 2 per cent sodium oxalate solution. Add 0.25 *N* sodium hydroxide solution drop by drop until the indicator begins to change, and 0.2 cc. of a 2.5 per cent solution of 8-hydroxyquinoline in glacial acetic acid. Complete as for macro samples making the final dilution to 10 cc.

<sup>139</sup> J. Lavollay, *Bull. soc. chim. biol.* 17, 432-8 (1935).

<sup>140</sup> See p. 297.

## CHAPTER XXVII

### NICKEL

#### NICKEL BY POTASSIUM THIOCARBONATE

WHEN nickel in ammoniacal solution is acted on by potassium thiocarbonate a rose red to dark red or brown color is produced, suitable for colorimetric estimation.<sup>1</sup> Metals such as copper and cadmium interfere but may be precipitated by hydrogen sulfide. Manganese, iron and cobalt present as a cobaltous compound, interfere. Cobalt is oxidized and the other two must be removed before applying the reagent. Zinc forms a white precipitate with the reagent but this is not present in sufficient quantity to cause interference if the work is carried out quickly. The separation of zinc from nickel is troublesome. If a rapid comparison is impossible add an amount of zinc to the standard similar to that in the sample. The sample should be chosen to contain less than 2.5 mg. of nickel. Conditions must be closely controlled to have the method quantitative but under proper control accuracy to less than 0.25 per cent of nickel in steel has been claimed. The nickel may be originally present as chloride, sulfate or nitrate in acid or neutral solution. A determination may be completed in one and a half hours. The potassium thiocarbonate solution may be kept for a week. In 20 cc., 0.17 mg. of nickel will give a dark red and 0.0008 mg., a light yellow. The reagent alone gives a yellowish color. The most accurate results are obtained between 0.02 and 0.1 mg. corresponding to 1 to 5 mg. of nickel in the sample dissolved.

**Procedure**--Dissolve 2 to 5 grams of sample with 50 cc. of 1:1 nitric acid. If necessary add 50 cc. of 1:1 sulfuric acid. If elements of the hydrogen sulfide group are present dilute to about 7 per cent acid, precipitate with hydrogen sulfide and filter. Oxidize the filtrate with excess bromine-water, neutralize and add concentrated ammonium hydroxide in slight excess. This precipitates manganese, iron and aluminum. Boil, cool, make up to 1 liter and filter through a dry filter into a dry receiver. Use 20 cc. of this solution for the comparison or a portion diluted to 20 cc. with a 1:50 ammonium hydroxide. To this add 0.5 cc. of a 4 per cent

<sup>1</sup> I. V. Lindt, *Z. anal. Chem.* 53, 165 (1914).

aqueous potassium thiocarbonate solution and mix. The color develops at once. The permanence of the color is questionable over a period of time. Above 0.17 mg. per cc. a precipitate forms in about 4 hours.

For the dilution or balancing methods add a suitable amount of standard nickel solution to a comparison cylinder. Dissolve 0.5 gram of ammonium nitrate in 1:50 ammonium hydroxide and add. If sulfuric acid was used in getting the sample in solution add 1 gram of ammonium sulfate. Dilute to 20 cc. with 1:50 ammonium hydroxide and add 0.5 cc. of 4 per cent aqueous potassium thiocarbonate solution. Mix and compare with a sample treated with the reagent at the same time. For dilution use 1:50 ammonium hydroxide.

For duplication add 0.5 gram of ammonium nitrate, with 1.0 gram of ammonium sulfate if sulfuric acid was used in dissolving the sample, to a comparison cylinder. Dissolve in 18 cc. of 1:50 ammonium hydroxide and add 0.5 cc. of the reagent. Add a standard nickel solution until a match is obtained. Adjust the volume to correspond to the sample and add a slight additional amount of standard solution if necessary.

**Standard**—Prepare a standard by dissolving 0.6730 gram of nickel ammonium sulfate,  $\text{NiSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ , in water and dilute to 1 liter. This solution contains 0.1 mg. of nickel per cc.

#### NICKEL BY DIMETHYLGLYOXIME

By the use of an oxidizing agent in an alkaline medium a red compound <sup>2</sup> of nickel and dimethylglyoxime is formed in solution and precipitation is prevented. The product is a complex in which nickel has a higher valence than 2, as shown by liberation of iodine on treatment with potassium iodide in acid solution, and by failure to form the sulfide.

Fairhall <sup>3</sup> believes that this method is only roughly quantitative. He finds that the color is due to crystalline particles of the nickel oxime which can be separated by filtration and that the method is turbidimetric rather than colorimetric. In that case the size of the particles varies with varying conditions so that closer control than usual in colorimetric methods is required. Copper must be absent.<sup>4</sup> The original qualitative method used lead dioxide as oxidizing agent. Bromine has been found preferable.

<sup>2</sup> F. Feigl, *Ber.* 57, 758 (1924).

<sup>3</sup> L. T. Fairhall, *J. Ind. Hyg.* 8, 528-33 (1926).

<sup>4</sup> A. P. Rollet, *Compt. rend.* 183, 212 (1926).

Satisfactory results have been obtained in the presence of 15 per cent of chromium.

The method is accurate to about 5 per cent between the limits of 0.001 and 0.01 mg. of nickel per cc. of solution. Working on a somewhat smaller scale with 0.9 mg. of nickel, accuracy to 0.2 per cent was obtained. By a modification of the procedure the nickel dimethylglyoxime is extracted with ether for estimation.<sup>5</sup> This permits a complete analysis of an alloy for nickel in less than 10 minutes and 1 per cent accuracy on 0.1–0.2 mg. of nickel.

**Sample—Cobalt salts.** For determination of nickel in cobalt salts, dissolve in water and convert the cobalt to cobalticyanide. If the ratio of cobalt to nickel is more than 1000:1, color the nickel standard to the same extent by addition of cobalticyanide from cobalt known to be free from nickel. Dilute to a suitable volume so that a 50 cc. aliquot will contain not over 0.5 mg. of nickel.

**Nickel steel.**—For determination in nickel steel dissolve a suitable sample of steel in nitric acid as usual. Neutralize the large excess of acid with ammonium hydroxide, leaving the solution faintly acid. Dilute to a volume such that 50 cc. contains less than 0.5 mg. of nickel.

**Alloys.**<sup>5</sup> Dissolve 10–15 mg. of the alloy in 10 cc. of concentrated nitric acid. Evaporate to about 5 cc. and dilute to 25 cc. with water.

**Biological Samples.**<sup>7</sup> Evaporate or dry the sample in a porcelain dish and ash. Add 10 cc. of concentrated hydrochloric acid and evaporate to dryness. Repeat 2 or 3 times. Extract the residue with 10 cc. of water containing 2 cc. of 4 *N* hydrochloric acid. If the sample contains iron in excess, such as a blood sample, treat the solution with excess ammonium hydroxide and filter. Repeat 3 times, redissolving the precipitate with the same quantity of acid as used for the extraction. If little or no iron is present sufficient must be added to combine with any phosphate. If phosphates are in excess neutralize the cold solution with 1:1 ammonium hydroxide, add 8 to 10 cc. of 10 per cent ammonium acetate solution and enough ferric chloride to tint the liquid yellowish-red. On boiling, the iron separates as phosphate and basic acetate. Wash the precipitate carefully. Precipitate the remaining iron with ammonium hydroxide as above.

Evaporate the united filtrates to dryness, filtering off iron if further precipitation occurs. Dissolve the residue in water and add 6 cc. of 4 *N*

<sup>5</sup> V. P. Ochotin and A. P. Suichov, *Z. anal. Chem.* 90, 109-11 (1932).

<sup>7</sup> H. W. Armit and A. Harden, *Proc. Royal Soc. (London)* 77B, 420 (1906).

hydrochloric acid. Pass hydrogen sulfide into the hot solution for half an hour. Let the sulfides settle, filter and wash with hydrogen sulfide solution. Evaporate the filtrate to dryness, redissolve in water and add 10 per cent sodium hydroxide solution until no more ammonia is evolved, avoiding excess. The nickel is precipitated as hydroxide. Convert to nickel sesquioxide by the addition of 1 to 2 cc. of bromine-water to the cold mixture. Filter the oxide, wash, dissolve in hydrochloric acid, evaporate to dryness and redissolve in 1:100 hydrochloric acid. Dilute to a volume such that 50 cc. contains less than 0.5 mg. of nickel.

**Procedure—Direct Comparison.** Take 50 cc. of solution containing 0.015 to 0.5 mg. of nickel. Add saturated bromine-water to distinct excess followed by sufficient 1:1 ammonium hydroxide to decolorize the excess of bromine but not to precipitate iron. Then add 0.5 cc. of a 0.1 per cent solution of dimethylglyoxime in 95 per cent alcohol and further amounts of 1:1 ammonium hydroxide sufficient to precipitate all the iron as ferric hydroxide. Biological samples will contain no iron at this point. Shake well and filter. Redissolve the precipitate in 1:1 hydrochloric acid and reprecipitate with addition of the reagent as above. Experiment has shown that all the nickel is in the filtrate.

Dilute both sample and standard to 100 cc. The sample and standard develop the color in 1 or 2 minutes. If the color is too faint, hasten development by heating sample and standard in the same water bath. It is probable that a maximum color is not reached, so that care must be taken to have standard and sample receive the same treatment at all times. Compare by dilution as soon as possible after preparation.

**Extraction.** Render the solution ammoniacal with 1:1 ammonium hydroxide and add sufficient excess to complete the transformation to the complex ammonium ions of copper and nickel. Heat and add 15 cc. of 1 per cent dimethylglyoxime in 95 per cent alcohol. Transfer to a separatory funnel, cool with water and add 15 cc. of ether. Shake and drain the aqueous solution from the ether solution. Wash the latter twice with water. Add 5 cc. of ethyl alcohol and 5 cc. of 4 per cent collodion solution. Dilute to a known volume with ether and compare with standards similarly treated.

**Standard**—As standard, dissolve 0.4479 gram of nickel sulfate,  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ , 0.6730 gram of nickel ammonium sulfate,  $\text{NiSO}_4 \cdot \frac{1}{2}\text{SO}_4 \cdot 6\text{H}_2\text{O}$ , or an equivalent amount of other nickel salt in water



and dilute to 1 liter. This contains 0.1 mg. of nickel per cc. and may be further diluted to a basis of 0.01 mg. per cc. if necessary.

Start the standard at the same time as the sample and add the same reagents to each, including a rough equivalent of the acid present if the sample is an acid solution.

#### NICKEL BY POTASSIUM DITHIOOXALATE

Potassium dithiooxalate is a very sensitive reagent for nickel.<sup>8,9</sup> The color in dilute solution is a deep magenta. The method is better than that with dimethylglyoxime or with  $\alpha$ -benzyloxime.<sup>10</sup> Beer's law applies over the range to which it is applied.

Manganese affects the color when present in relatively large amounts; 15 mg. in 50 cc. just begins to show color. In biological material, for which the method was developed, it is not usually present in quantities great enough to interfere. Iron must be removed, as it gives a deep purple. Cobalt must be absent, or a special procedure used. Other ions which give color are antimony, bismuth, cadmium, cobalt, copper, mercury, silver, tin, palladium, platinum, zinc, cerium, gold, thallium, titanium and vanadium. Calcium, magnesium, barium, strontium, aluminum and phosphates need not be absent. The color develops in either neutral or acid solution. The acidity may vary from 0.0001 molar to 0.1 molar of hydrochloric or acetic acid. If the nickel is less than 0.05 mg., an acidity over 0.01 molar gives a yellowish brown instead of pink. An average error of 2 per cent was reported for 1 mg. of nickel with a maximum error of about 5 per cent.

**Sample**—Dry the sample. Transfer to a porcelain dish, char and ash, the latter preferably in a muffle furnace at low red heat. In evaporating urine or milk to dryness add 10 cc. of concentrated hydrochloric acid to prevent bumping. Do not ash over one-half hour or at too high a temperature as the salts will fuse and coat the carbon. Let cool, treat with 15 cc. of 1:1 hydrochloric acid and filter. Cover the residue with water and heat to boiling, covering with a watch glass. Filter and extract twice more with boiling water. Return the filter paper and residue to a porcelain dish, dry and ash again in the muffle. When cold, extract as before.

<sup>8</sup> H. O. Jones and H. S. Tasker, *J. Chem. Soc.* 95, 1904-9 (1909).

<sup>10</sup> John H. Yoe and Floyd H. Wirsing, *J. Am. Chem. Soc.* 54, 1866-76 (1932).

Usually at least three ignitions and extractions are required before all the nickel goes into solution. A siliceous residue may be discarded.

Neutralize the combined acid solutions with 1:1 ammonium hydroxide, using methyl orange as an indicator. Add a few drops of hydrochloric acid until just acid. Saturate cold with hydrogen sulfide and let stand until precipitation is complete. Filter and wash the residue with hydrogen sulfide solution. Boil the filtrate until free of hydrogen sulfide and add bromine-water to oxidize iron to the ferric state.

*Cobalt Absent.* Iron must be eliminated. Phosphate must be added if absent. Treat the cold solution with 10 cc. of 50 per cent ammonium acetate solution and 0.5 cc. of glacial acetic acid. Unless the solution is cold before ammonium acetate is added some iron will be reduced. Filter from the ferric phosphate into a volumetric flask. The filtrate should be water-white and perfectly clear. Dilute to 500 cc.

*Cobalt Present.* Add to the solution 10 cc. of a 20 per cent sodium citrate solution. Then add 5 cc. of saturated ammonium oxalate solution very slowly with stirring. Calcium oxalate is precipitated. Next add 1:1 ammonium hydroxide to precipitate ammonium magnesium phosphate in the same solution. After the calcium and magnesium are quantitatively precipitated, filter, wash, redissolve in hydrochloric acid and precipitate as before. Filter and combine the two filtrates. Treat with an excess of  $\alpha$ -benzylidioxime and filter. Dissolve the residue in *aqua regia* and evaporate to dryness in a porcelain dish. Add 2 cc. of concentrated hydrochloric acid and again evaporate to dryness to drive off excess nitric acid. Dissolve the residue in a 0.5 cc. of 1:1 hydrochloric acid and dilute to 500 cc.

**Reagent**—The reagent is obtainable commercially. Fairhall<sup>3</sup> has given the following method of preparation.

Treat 2 moles of ethyl mercaptan with 1 mole of oxalyl chloride at room temperature, adding the latter in small portions. After most of the hydrogen chloride has escaped heat gently to drive off the remainder and any excess of either reacting substance. Dissolve the resulting ethyl dithiooxalate in alcohol and add an equivalent of potassium hydrosulfide in alcohol. To prepare the latter treat an equivalent weight of potassium hydroxide in alcohol with hydrogen sulfide until the cold solution is saturated with the gas, let stand over night and filter. Pour the alcoholic potassium hydrosulfide into the solution of ethyl dithiooxalate, stirring vigorously, and let stand an hour. Collect the white crystalline potassium

dithiooxalate on a Büchner funnel and wash with cold 95 per cent alcohol. Dry and store in amber colored bottles. The reagent is stable for several months, but finally darkens and produces a turbidity which prevents accurate readings.

**Procedure**—Transfer 50 cc. to a comparison tube and add 1 cc. of 0.1 per cent aqueous potassium dithiooxalate solution. A brownish tint indicates excess cobalt; purple indicates iron. In the latter case repeat the oxidation and precipitation with an aliquot.

Compare with a standard prepared by addition of known amounts of nickel<sup>11</sup> to distilled water containing the same amounts of acid and salts as the sample. In the absence of cobalt this will be 1 gram of ammonium chloride, 1 cc. of 50 per cent ammonium acetate solution and 1 drop of glacial acetic acid to 50 cc. of standard. In the presence of cobalt only 1 gram of ammonium chloride and 1 drop of 1 : 1 hydrochloric acid need be added. A suitable series contains 0.005, 0.01, 0.02, 0.03, 0.04 and 0.05 mg. of nickel.

The color changes at a variable rate with different lots of reagent. This is not significant with less than 0.05 mg. of nickel in 50 cc. and low acidity. In strongly acid solutions the color change is noticeable after an hour or more when turbidity appears.

#### NICKEL BY THE FORMALDOXIME REAGENT

The reaction of nickel with a formaldoxime reagent<sup>12</sup> may be used for its estimation in cobalt salts.<sup>12</sup> A brownish tint is produced. The method will detect 0.1 per cent of nickel in a cobalt salt.

**Reagent**—Mix 7 grams of hydrazine hydrochloride, 15 cc. of water and 3 grams of trioxymethylene. Heat to boiling slowly, boil until clear and cool.

**Procedure**—Prepare a solution of the cobalt salt sample to contain 50 mg. of cobalt per liter. Prepare a similar standard of known nickel-free salt. Transfer 20 cc. of these solutions to a pair of tubes.

<sup>11</sup> See p. 314.

G. Denigès, *Bull. soc. pharm. Bordeaux* 70, 101-7 (1932).

To each add 2 drops of the reagent and mix. Add 4 drops of 40 per cent sodium hydroxide solution and mix. The nickel-free solution will be pale yellow. If the sample contains nickel it will be brown. Compare with a series of standards prepared from cobalt salt solution to which known amounts of nickel <sup>13</sup> were added.

#### NICKEL AS THE SULFIDE

In the absence of other metals giving colored sulfides the general method should be applicable to nickel. No detailed method is available.

With sodium sulfide as the precipitating agent a saturated solution of basic nickel hydrosulfide contains  $0.8 \times 10^{-5}$  per cent of nickel.<sup>14</sup> The sulfide with excess hydrogen sulfide present has the formula  $\text{Ni}(\text{OH})\text{SH}$ .

---

<sup>13</sup> See p. 314.

<sup>14</sup> Alexander Mickwitz, *Z. anorg. allgem. Chem.* 196, 113-9 (1931).

## CHAPTER XXVIII

### COBALT

#### COBALT AS THE CHLORIDE IN CONCENTRATED HYDROCHLORIC ACID

THE determination of cobalt in nickel or its alloys <sup>1</sup> by the color of the chloride in concentrated hydrochloric acid has been carefully studied. In this medium cobalt chloride gives a dark blue color, nickel chloride an intense yellow, and the combination of the two a green. The amount of nickel may vary over a considerable range without changing the green color produced by a definite weight of cobalt. The method is suitable for nickel alloys whose cobalt content is between 0.1 and 10 per cent. From 0.2 to 1.9 mg. per cc. can be estimated with an accuracy of 2 per cent.<sup>2</sup>

The same concentration of hydrochloric acid should always be used; the usual specific gravity of 1.19 is recommended. The color of the acid solution is destroyed by nitric acid and by free chlorine. For this reason several evaporations with concentrated hydrochloric acid are necessary. Some foreign chlorides, especially those of copper and iron, also alter the color.

Cobalt and nickel can frequently be separated from other metals by electrolysis in ammoniacal solution and the determination carried out on a solution of this deposit.

A maximum error of 5 per cent is reported. Cobalt may be determined similarly in many other metal alloys by use of a standard solution of the corresponding metal. If the principal metal present furnishes a colorless solution, as in the case of lead, zinc, cadmium, tin, bismuth and several others, the solutions of their cobalt alloys exhibit a pure blue color in the absence of interfering metals noted above.

**Sample—Copper and Iron Absent.** Dissolve 0.2 gram of alloy in 5 cc. of concentrated nitric acid. Evaporate to dryness on a water bath and take up with 5 cc. of concentrated hydrochloric acid. Repeat this operation twice more. Dissolve the residue in 5 cc. of 1:1 hydrochloric acid,

<sup>1</sup> C. Hüttner, *Z. anorg. Chem.* **86**, 341 (1914).

<sup>2</sup> Wilhelm Heinz, *Z. anal. Chem.* **78**, 427-39 (1929).

transfer to a Nessler tube and dilute to 100 cc. with concentrated hydrochloric acid.

*Copper and Iron Present.* Dissolve 0.2 gram of alloy as above and evaporate to dryness on the water bath. Take up with 5 cc. of concentrated hydrochloric acid and repeat the evaporation. Take up with 5 cc. of 1:4 hydrochloric acid and dilute to 50 cc. with water.

Precipitate copper by hydrogen sulfide in hot solution. Filter, boil off hydrogen sulfide and oxidize with 1 drop of concentrated nitric acid. Evaporate to dryness on the water bath. Take up the residue with a few drops of 1:40 hydrochloric acid. Neutralize the clear solution with 1 per cent sodium carbonate solution and add 1 cc. of 10 per cent sodium acetate solution while hot. Boil for 10 minutes. Let the precipitate of basic ferric acetate settle, filter and wash with hot water. Add 5 cc. of concentrated hydrochloric acid to the filtrate and evaporate to dryness.

Dissolve the residue in 5 cc. of 1:1 hydrochloric acid, transfer to a Nessler tube and dilute to 100 cc. with concentrated hydrochloric acid. The sodium chloride formed does not interfere.

**Procedure**—Into a similar tube introduce 90 cc. of standard nickel solution and concentrated hydrochloric acid. If the alloy contains over 90 per cent of nickel the standard solution is used without dilution. Add standard cobalt solution from a buret until the green color in the two tubes has the same quality. Adjust the volume of the standard to 100 cc. with suitable amounts of standard nickel and cobalt solutions and concentrated hydrochloric acid. The number of cc. of cobalt solution used gives the per cent of cobalt present.

**Standards**—Prepare standard solutions of cobalt and nickel containing 2 grams of metal in 1 liter of hydrochloric acid. In one case dissolve 4.9557 grams of nickel nitrate,  $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , and in the other case 4.9362 grams of cobalt nitrate,  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , in 25 cc. of 1:1 hydrochloric acid. Convert these to the chlorides by several evaporations with 25 cc. of concentrated hydrochloric acid. Finally dissolve in 25 cc. of 1:1 hydrochloric acid and dilute to 500 cc. with concentrated hydrochloric acid. Each cc. contains 2 mg. of cobalt or nickel.

#### COBALT BY $\alpha$ -NITROSO- $\beta$ -NAPHTHOL

The orange to red color produced by cobalt with  $\alpha$ -nitroso- $\beta$ -naph-

thol<sup>3,4</sup> may be used for the quantitative determination of small amounts in varnishes, paints and similar commercial products.<sup>5</sup> Iron, aluminum and chromium are kept in solution by addition of ammonium citrate. More than a trace of copper and any considerable amount of nickel or manganese interfere. They can be removed prior to addition of the reagent. The alkalinity of solutions compared should be approximately the same. One p.p.m. in the solution examined is easily detected. The accuracy by this method should be better than 5 per cent. One investigator has reported the method unsatisfactory for accurate work.<sup>6</sup>

**Sample**—Ash a suitable sample of paint, varnish or other organic matter. To about 0.1 g. of ash add 5 cc. of 1:1 hydrochloric acid. If any undissolved residue remains, decant. Add 3 cc. of concentrated hydrochloric acid and 1 cc. of concentrated nitric acid to the insoluble matter. Filter and wash any residue of silica.

Evaporate to dryness on a water bath. If nitric acid was used above, add 2 cc. of concentrated hydrochloric acid and again evaporate to dryness. Dissolve in 25 cc. of 1:10 hydrochloric acid. If necessary dilute to a definite volume and take a suitable aliquot.

**Removal of Copper.** If copper is present it is removed by precipitation with hydrogen sulfide in acid solution. Dilute the above solution to 150 cc. and saturate with hydrogen sulfide. After precipitation is complete, filter and concentrate by evaporation to about 25 cc.

**Removal of Nickel.** If necessary to remove nickel, add 10 cc. of ammonium citrate solution to the solution of the sample. Heat and add a slight excess of a 1 per cent alcoholic solution of dimethylglyoxime. Add ammonium hydroxide until the liquid is slightly alkaline. Filter and wash. Evaporate the filtrate to dryness and ignite carefully to remove excess dimethylglyoxime. Treat the residue with 3 cc. of concentrated hydrochloric acid and 1 cc. of concentrated nitric acid. Evaporate to dryness on the water bath, add 2 cc. of concentrated hydrochloric acid and again evaporate to dryness. Dissolve in 25 cc. of 1:10 hydrochloric acid.

**Removal of Manganese.** If the manganese is not in excess of the cobalt it may be ignored. If necessary to remove manganese add 25 cc. of 1:1

<sup>3</sup> M. Jlinski and G. von Knorre, *Ber.* 18, 699-704 (1885); *Z. anal. Chem.* 24, 595-8 (1885).

<sup>4</sup> F. W. Atack, *J. Soc. Chem. Ind.* 34, 641 (1915).

<sup>5</sup> E. G. Jones, *Analyst* 43, 317 (1918).

<sup>6</sup> Wilhelm Heinz, *Z. anal. Chem.* 78, 427-39 (1929).

nitric acid to the solution. Add 0.1 g. of sodium bismuthate. Digest until the permanganate color disappears and the manganese is precipitated. Filter and evaporate the filtrate to dryness on a water bath. Treat with 2 cc. of concentrated hydrochloric acid, again evaporate to dryness, and dissolve the residue in 25 cc. of 1:10 hydrochloric acid.

**Reagents**— *$\alpha$ -Nitroso- $\beta$ -naphthol*. Mix 1 cc. of *N* sodium hydroxide solution with 20 cc. of water. Add 0.1 gram of  $\alpha$ -nitroso- $\beta$ -naphthol and boil. Cool, filter and dilute to 200 cc. This solution keeps indefinitely.

*Ammonium Citrate Solution*. Dissolve 500 grams of citric acid in 250 cc. of water and add 500 cc. of concentrated ammonium hydroxide.

**Procedure**—Transfer the sample to a Nessler tube and add 5 cc. of ammonium citrate solution. Dilute to 95 cc. with distilled water. Add 5 cc. of  $\alpha$ -nitroso- $\beta$ -naphthol reagent and mix.

As standard mix 25 cc. of 1:10 hydrochloric acid, 10 cc. of ammonium citrate solution and distilled water to make 90 cc. Add 5 cc. of reagent, mix well and add stock cobalt solution until the color of the sample is duplicated. Adjust the volume to 100 cc. For dilution or balancing add a suitable volume of the cobalt solution to the standard before diluting to 100 cc. The comparison should be carried out as quickly as possible, as a red precipitate appears after a short time.

**Standard**—As standard dissolve 0.4936 gram of cobalt nitrate,  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , in water and dilute to 1 liter. This contains 0.1 mg. of cobalt per cc.

#### COBALT AS COBALTAMMINE

If cobalt is oxidized in ammoniacal solution the yellow color changes to an intense rose pink in the course of 1 or 2 minutes. The pink color, which is produced by the cobaltamine is proportional to the amount of cobalt present.<sup>7</sup> Any iron present will be precipitated and may be removed by filtration. The presence of nickel gives rise to a blue color with excess ammonium hydroxide, varying from purple to greenish blue according to the concentrations of ammonium hydroxide and nickel. In spite of this, cobalt may be estimated in the presence of nickel but a special procedure is necessary. Manganese is precipitated as manganese dioxide and removed by filtration.

<sup>7</sup> B. S. Evans, *Analyst* 50, 389 (1925).



The usual error reported is about 2 per cent. In the presence of nickel it is about 6 per cent for 1 mg. of cobalt. In steel containing 0.5 per cent of cobalt 2 per cent error was found, both with and without nickel present.

**Procedure—Nickel Absent.** Dissolve 1 gram of sample in 25 cc. of distilled water. Samples of minerals or ores dissolved in acid by previous procedures may be evaporated to dryness on the water bath in order to meet the conditions of this method. Transfer to a Nessler tube. Add 2 cc. of 20 per cent ammonium chloride solution and 1 : 1 ammonium hydroxide until the solution contains a slight excess of ammonium hydroxide and the precipitated cobalt has redissolved. About 2–5 cc. will usually be required. Dilute to about 95 cc.

In a second Nessler tube put the same quantities of ammonium chloride and ammonium hydroxide solutions. Dilute to about 90 cc. with water and add approximately 0.6 gram of sodium peroxide to each tube. To the second tube add a standard cobalt solution, matching the colors in the same volume. By adding cobaltamine instead of cobalt solution the delay before full development of color may be avoided.

**Nickel Present.** Use a Walpole colorimeter, or a colorimeter constructed to hold 4 tubes, two of them, A and B, being above and in line with the two below, C and D respectively. In looking through the longitudinal depths the sum of A and C on one side is compared with B and D on the other.

Dissolve a 2 gram sample in 50 cc. of water, after evaporating excess acid if necessary. Dilute to exactly 100 cc. and put 50 cc. in each of tubes A and B. To each add 2 cc. of 20 per cent ammonium chloride solution, 2 cc. of 1 : 1 ammonium hydroxide and about 5 grams of sodium citrate to help produce a pure blue cobalt tint. For the same reason the amount of excess ammonium hydroxide is kept low. To tube A add about 0.6 gram of sodium peroxide. Into each of the tubes C and D put 5 cc. of 20 per cent ammonium chloride solution, 5 cc. of 1 : 1 ammonium hydroxide and about 5 grams of sodium citrate. Dilute the contents of C so that the volume will be the same as in A and B. To tube D add about 0.6 gram of sodium peroxide and a standard cobalt ammonium sulfate solution until the colors match with the same volumes in all the tubes.

**Steel.** Dissolve a 5 gram sample in 20 cc. of *aqua regia*. Add about 100 cc. of water. Nearly neutralize excess acid with ammonium hydroxide. Add 5 cc. of concentrated zinc oxide paste, dilute to 500 cc. and filter through a dry filter. Measure 150 cc. of filtrate into a dry beaker and add 7.5 cc. of 20 per cent ammonium chloride solution, 22.5 cc. of 1 : 1 am-

monium hydroxide and about 0.6 gram of sodium peroxide. Filter to remove manganese dioxide and put 120 cc. of filtrate into tube A of the colorimeter described for solutions containing nickel. This corresponds to a 1 gram sample.

To B, C and D add 5 cc. of 20 per cent ammonium chloride solution, 5 cc. of 1:1 ammonium hydroxide, and 20 cc. of water. To A and B add 5 grams of sodium citrate, to D add 0.6 gram of sodium peroxide. Match the color by adding a nickel solution to C and a standard cobalt solution to D, having the same volumes of solution present in all 4 tubes at the end. If nickel is absent in the sample, tubes C and D need not be used.

**Standard Solutions—Cobalt.** Dissolve 6.70 grams of cobalt ammonium sulfate in water and dilute to 1 liter. Each cc. of this solution corresponds to 1 mg. of cobalt. This solution is perfectly stable.

**Cobaltammine.** Place 20 cc. each of 20 per cent ammonium chloride solution and of 1:1 ammonium hydroxide in a 200 cc. volumetric flask. Add about 0.6 gram of sodium peroxide. Add 50 cc. of the standard cobalt solution 1 cc. at a time, allowing the pink color to develop after each addition. Add more sodium peroxide after every 3 or 4 cc. Dilute to 200 cc., shake, and let stand several hours before use. This may be used to compare directly with the unknown after the color has been developed in the latter. This solution is stable for some months.

#### COBALT BY POTASSIUM FERRICYANIDE IN AMMONIACAL SOLUTION

Cobalt salts give a deep red color with potassium ferri cyanide in ammoniacal solution.<sup>8</sup> The method has been applied successfully to steel containing both nickel and chromium. Amounts of cobalt added of the order of 0.02 to 0.04 per cent were accurately recovered.

**Procedure—**Dissolve 1 gram of steel filings in 10 cc. of *aqua regia*. Evaporate to dryness and ignite the residue to eliminate nitrates. Dissolve in 25 cc. of 1:1 hydrochloric acid. Evaporate to about 5 cc. and dilute with 25 cc. of water. Precipitate iron and chromium by addition of 10 cc. of a 10 per cent emulsion of zinc oxide. Dilute to 200 cc. and filter. To 100 cc. of filtrate add 5 cc. of concentrated hydrochloric acid. Pass in hydrogen sulfide to precipitate copper, and filter off the copper sulfide. Boil the filtrate to remove hydrogen sulfide. Add 0.2 gram of

$\alpha$ -nitroso- $\beta$ -naphthol dissolved in 10 cc. of glacial acetic acid. Boil a few minutes and let stand in a warm place for a half hour.

Filter off the precipitate of cobalt- $\alpha$ -nitroso- $\beta$ -naphthol. Wash with 1:4 hydrochloric acid and with hot water. Ignite in a porcelain crucible in a muffle at about 800°. Dissolve the residue in 10 drops of hot concentrated hydrochloric acid, dilute with 10 cc. of water, and filter if necessary, into a comparison tube. Add 10 cc. of 1:1 ammonium hydroxide and 5 cc. of a 0.1 per cent solution of potassium ferricyanide.

Into the standard tube put 10 cc. of 1:1 ammonium hydroxide, 0.5 cc. of concentrated hydrochloric acid, and 5 cc. of the potassium ferricyanide solution. Add a standard solution equivalent to 1 mg. of cobalt per cc.,<sup>9</sup> until the colors match. The sample matched is 0.5 gram.

If the cobalt is over 0.1 per cent add 5 cc. of reagent for each 0.1 per cent present, to both sample and standard.

#### COBALT BY DIMETHYLGLYOXIME

Cobalt in solution gives a distinct brown color with dimethylglyoxime when only 0.0005 mg. per cc. is present.<sup>10</sup> Iron and copper must both be removed. The accuracy is 5 to 10 per cent.

**Procedure**—Prepare the solution as for the determination of nickel with dimethylglyoxime.<sup>11</sup> Acidify slightly with 1:1 hydrochloric acid. Add 5 cc. of 10 per cent sodium acetate solution and heat to boiling. Add a slight excess of dimethylglyoxime reagent<sup>12</sup> and dilute to 100 cc. If nickel is present, filter. Let cool slowly and compare with a standard cobalt solution heated and treated with the reagent at the same time as the sample. The color varies slightly with variation in the rate of cooling.

#### COBALT BY AMMONIUM THIOCYANATE

An estimation may be made by treating a solution of cobalt with ammonium thiocyanate and extracting with amyl alcohol.<sup>13</sup> The blue color is due to cobalt ammonium thiocyanate,  $(\text{NH}_4)_2\text{Co}(\text{CNS})_4$ . Nickel does

<sup>9</sup> See p. 326.

<sup>10</sup> S. A. Braley and F. B. Hobart, *J. Am. Chem. Soc.* 43, 482 (1921).

<sup>11</sup> See pp. 315-6.

<sup>12</sup> See p. 316.

<sup>13</sup> A. D. Powell, *J. Soc. Chem. Ind.* 36, 273-4 (1917).

not form a thiocyanate<sup>14</sup> and iron may be prevented from interfering by phosphate.<sup>15</sup>

By addition of acetone the color may also be estimated in the original solution if the amount of cobalt in 20 cc. is between 0.04 and 45 mg.<sup>16</sup> The method has been reported as unsatisfactory for accurate and convenient work.<sup>17</sup>

phate to 20 cc. of the cobalt solution. This pr  
Add 5 cc. of 60 per cent ammonium thiocya  
50 cc. with acetone. Compare the color with that of a known cobalt solu-  
tion<sup>18</sup> diluted to 25 cc. with water and similarly treated. If dilution is  
resorted to for comparison, do not have the standard vary from the  
sample by more than 50 per cent, and carry out the dilution of sample  
or standard with 50 per cent acetone solution.

*By*

cobalt solution. Add 2:	per	
mix well and extract twice	10 cc. of amyl alcohol.	two ex-
s and compare, by dil	with amyl alcohol,	
ion <sup>18</sup> similarly prepar	'he	
red due to adsorption of color by the filter paper.		

#### COBALT BY REDUCTION OF ARSENOPHOSPHOTUNGSTIC

is reaction cobalt can be detected in 300–400 times its weight of  
ated in the presence of 2.5–5.0 mg. of arsenic  
reduction product of tungsti

	ganous, mercurous, c
terfer	ount of cobalt is pr
nesium,	barium, tin, antimony, arsenic,

E. A. Lum, *Chem. News* 141, 140 (1930).

<sup>15</sup> J. W. Mellor, *Trans. Eng. Ceram. Soc.* 8, 132 (1910).

<sup>16</sup> E. S. Tomula, *Acta Chem. Fennica* 2, 72–80 (1929); *Z. anal. Chem.* 83, 6–14 (1931).

<sup>17</sup> Wilhelm Heinz, *Z. anal. Chem.* 78, 437–9 (1929).

<sup>18</sup> See p. 324.

<sup>20</sup> Abraham Leiberson, *J. Am. Chem. Soc.* 52, 464–5 (1930).

muth, lead or silver ions it tends to produce turbidity. Manganese and chromium interfere with the color readings.

**Reagent**—Dissolve 100 grams of sodium tungstate in 600 cc. of water in a liter Pyrex flask. Add 50 grams of pure arsenic oxide, 25 cc. of syrupy phosphoric acid and 20 cc. of concentrated hydrochloric acid. The latter serves as a condensing agent. Boil for 20 minutes, cool and dilute to 1 liter. The reagent keeps indefinitely.

**Procedure**—Add 3 cc. of the reagent to 10 cc. of approximately neutral solution of the sample and to 1 cc. of standard and 9 cc. of water. Invert both tubes once to mix and add to each 4 cc. of a 5 per cent solution of sodium cyanide containing 2 cc. of concentrated ammonium hydroxide per liter. Invert the two tubes simultaneously and compare between 2 and 10 minutes after mixing. No turbidity will appear within 10 minutes if not over 5 mg. of nickel are present. More cyanide will decolorize more nickel but turbidity appears more quickly.

**Standard**—Dissolve 1.25 gram of cobalt chloride dihydrate,  $\text{CoCl}_2 \cdot 2\text{H}_2\text{O}$ , 2.02 grams of cobalt chloride hexahydrate,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , or 2.47 grams of cobalt nitrate,  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , in water and dilute to 1 liter. Each cc. contains 0.5 mg. of cobalt.

#### COBALT BY OXIDATION OF THE COBALT-CYSTEINE COMPLEX

Cobalt sulfate and cysteine form a complex which is oxidized by air. This gives a yellow to brown color similar in tint to that of ammonia with Nessler's reagent and suitable for colorimetric estimation.<sup>21</sup> Reasonable amounts of nickel, copper, manganese and iron do not interfere. An amount of nickel double that of the cobalt alters the tint appreciably.

**Sample**—Place a sample containing not more than 5 mg. of cobalt in a platinum crucible with about 1.5 cc. of concentrated sulfuric acid. Set this in a large nickel crucible and heat to dryness. All organic matter is destroyed and cobalt is present as the sulfate.

**Procedure**—To the sample add 75 cc. of solution buffered to pH 7.5. For details of buffers see the chapter on hydrogen-ion.<sup>22</sup> When dissolved,

<sup>21</sup> L. Michaelis and S. Yamaguchi, *J. Biol. Chem.* 83, 367-73 (1929).

<sup>22</sup> See pp. 671-86.

and filtered if necessary, add about 10 mg. of cysteine hydrochloride crystals. Gently mix this solution. Oxidation by air will complete the conversion of the cobalt complex to the colored compound. Compare with a standard prepared at the same time from a known amount of cobalt sulfate dissolved in the properly buffered solution. For very small amounts of cobalt reduce the quantities of all materials proportionately.

#### COBALT BY HYDROGEN PEROXIDE IN ALKALINE SOLUTION

Cobalt produces the green color of a hydrocarbonate,  $\text{Co}(\text{OH})_4(\text{CO}_2\text{H})_2$  with hydrogen peroxide in the presence of potassium bicarbonate or ammonium bicarbonate.<sup>23</sup> The method has been developed for indirect estimation of potassium precipitated as cobaltinitrite. The reaction is specific for cobalt and very sensitive. It will permit the determination of 4 mg. of cobalt per liter. If the solution is too concentrated precipitation occurs. In dilute solution no change occurs on standing for weeks. Alkali chlorides up to 80 grams per liter do not affect the color. The maximum sensitivity is obtained over the range 0.001–0.0075 *M*. The accuracy is within 5 per cent. Results are slightly higher than by permanganate titration of the cobaltinitrite.

**Sample**—Prepare by the usual procedure, free from organic matter.

*Cobaltinitrite*. Decompose the precipitate by dissolving in 1:1 hydrochloric acid and evaporate to dryness. This drives off the nitrite fumes and converts cobalt to the chloride. Dissolve in water.

**Procedure**—Put 10 cc. of sample in a 50 cc. volumetric flask. Add 0.5 cc. of 3 per cent hydrogen peroxide and dilute nearly to the mark with a saturated solution of potassium bicarbonate or ammonium bicarbonate. Mix well and complete the dilution with bicarbonate solution or with distilled water. Mix and compare with a standard similarly treated.

#### COBALT AS THE SULFATE

Direct comparison of cobalt sulfate with a standard has been reported to give results accurate to 2.2 per cent within the range 0.4–3.8 mg. per cc.<sup>2</sup> Other colored salts must be absent.

## COBALT AS THE SULFIDE

Estimation of cobalt as the basic hydrosulfide,  $\text{Co}(\text{OH})\text{SH}$ , as precipitated by sodium sulfide solution has not been worked out in detail. The saturated solution contains  $0.3 \times 10^{-5}$  per cent of cobalt.<sup>25</sup> With excess hydrogen sulfide the absorption shows an inflexion at  $\text{Co}(\text{SH})_2$  with a solubility of  $1.0 \times 10^{-5}$  per cent cobalt. The operation would be carried out as is usual for sulfides, with other ions producing colored sulfides absent.

## CHAPTER XXIX

### MANGANESE

#### MANGANESE AS PERMANGANATE

and per-  
of a  
lead dioxide  
gent. As excess lead dioxide  
mentation its use is not very  
... largely replaced by potas-  
sium or a necessary catalyst for the  
is first the formation of silver  
ganat  
probably the rely r  
true ot always obtained and oxidation may be  
inco: sodium bismuthate is reported <sup>8</sup> to lead to  
with minute amounts of manganese, but  
reagent must be removed before compari-  
to be free from the faults of the

<sup>1</sup> W. Crum, *Ann.* 55, 219 (1845).

<sup>2</sup> P. Pickard, *Compt. rend.* 75, 1821 (1872).

<sup>3</sup> G. Bertrand, *Bull. soc. chim.* [4] 9, 361-70 (1911)

<sup>4</sup> A. A. Blair, "The Chemical Analysis of Iron," 8th ed., p. 124. J. B. Lippincott Co., Philadelphia, Pa. (1918).

. Marshall, *Chem. News* 83, 76 (1901).



vegetable products was fully recovered.<sup>11</sup> It is desirable that, for very small amounts of manganese, the acidity of sample and standard be very similar. At 5 to 6 per cent of sulfuric acid the color is stable. An excess causes incomplete development or rapid fading. The estimation of 0.01 mg. of manganese in 50 cc. of solution is satisfactory.<sup>12</sup>

Comparison against an inverted V of white paper improves the accuracy of estimation. By using this technique, 0.001 mg. per 50 cc. can be detected, and below 0.01 mg. estimated, to 0.001 mg.<sup>13</sup> When applied to plant materials, the periodate and persulfate methods were equally satisfactory. The bismuthate method gave low results.<sup>14</sup> The persulfate method is recommended for analysis of pure aluminum.<sup>15</sup>

By electrolysis of an acid solution of a mineral in special apparatus, manganese present as not over 0.1 per cent can be estimated.<sup>16</sup> Reading in the photoelectric colorimeter<sup>17</sup> and persulfate micro estimation<sup>17a</sup> have been recommended.

Another method of oxidation is based on the solubility of manganous hydroxide in glycerol and the readiness with which such a solution is oxidized by the action of air or of sodium hypochlorite.<sup>18</sup> The use of an alkaline potassium hypochlorite solution in the presence of copper sulfate has been suggested<sup>19</sup> as has also hydrogen peroxide.<sup>20</sup>

Methods involving reactions other than oxidation to permanganate, such as precipitation in an alkaline tartrate solution by a ferrocyanide,<sup>21</sup> leading to a comparison of turbidity rather than color, have not been developed to any great extent. Color reactions with tetramethyldiamidodiphenylmethane and with potassium iodide and starch have been reported to be unreliable.<sup>22</sup>

When the color comparison is made the solutions should not contain more than 2 mg. of manganese per 100 cc. otherwise the color is too deep

<sup>11</sup> Marion B. Richards, *Analyst* 55, 554-60 (1930).

<sup>12</sup> J. T. Skinner and W. H. Peterson, *J. Biol. Chem.* 88, 347-51 (1930).

<sup>13</sup> Norman Ashwell Clark, *Ind. Eng. Chem., Anal. Ed.* 5, 241-3 (1933).

<sup>14</sup> Jehiel Davidson and Ruth G. Capen, *J. Assoc. Official Agr. Chem.* 14, 547-51 (1931).

<sup>15</sup> F. Pavelka and Hermine Morth, *Mikrochemie* 13, 305-12 (1933).

<sup>16</sup> K. A. Nenadkevich, *Compt. rend. acad. sci.* 1931A, 153-9.

<sup>17</sup> M. Bender and H. Hirschmüller, *Z. anal. Chem.* 92, 1-7 (1933).

<sup>17a</sup> I. M. Korenman, *Microchemie* 15, 289-94 (1934).

<sup>18</sup> N. Tarugi, *Gazz. chim. ital.* 36, 332-47 (1906).

<sup>19</sup> M. Duyk, *Ann. chim. anal.* 12, 465 (1907).

<sup>20</sup> J. Heslinga, *Chem. Weekblad* 11, 302 (1922).

<sup>21</sup> F. Croner, *Gesundh. Ing.* 28, 197-8 (1905).

<sup>22</sup> D. H. Wester, *Rec. trav. chim.* 39, 414-22 (1920).

for accurate reading. The errors reported vary from 1 to 7 per cent, the latter for exceedingly small amounts of manganese. The usual estimate is from 2 to 3 per cent. Chromium interferes only when present in large quantities. Interference by iron is prevented by phosphates.

The manganese content of flour and bread has been suggested as a more satisfactory criterion than ash or protein determinations for detecting the addition of milling by-products.<sup>25</sup> The manganese is much more concentrated in the hull.<sup>26</sup>

**Sample—Pig Iron.** To 50 to 200 mg. of sample add 10 cc. of 1:1 nitric acid and heat gently until decomposition is complete.<sup>27</sup> Add about 0.5 gram of moist ammonium persulfate and continue heating until the carbon is completely oxidized. The persulfate should have been moistened a day or two before use as persulfate oxidation does not proceed smoothly when the added salt is dry.<sup>28,28a,28b</sup> This effect is probably due to the small solubility of the salt. If necessary filter to remove silica or graphitic carbon and wash the filter well.

**Steel.**<sup>29</sup> If the steel contains more than 0.75 per cent of manganese use a sample of 0.1 gram, otherwise 0.2 gram. Transfer to a flask, add 10 cc. of 1:1 nitric acid and heat on the water bath until dissolved.

The solution in which carbon has been estimated colorimetrically may also be used as sample,<sup>30</sup> diluting to 20 cc. if the manganese content is under 0.8 per cent or to 25 cc. if it is over that.

**Alloy Steels.**<sup>31</sup> Treat 1 gram of sample with 35 cc. of 1:6 sulfuric acid and heat until the action ceases. Add about 0.5 gram of ammonium persulfate in 10 cc. of water and boil until the excess persulfate is decomposed. Cool to room temperature, dilute to about 100 cc. and nearly neutralize with concentrated ammonium hydroxide. Transfer to a 250 cc. volumetric flask and add excess zinc oxide emulsion. Dilute to the mark

<sup>25</sup> P. Bruère, *Ann. fals.* 27, 150-7 (1934).

<sup>26</sup> P. Bruère. *Compt. rend.* 198, 504-6 (1934).

<sup>27</sup> The Chemists' Committee of the U. S. Steel Corp., "Methods for the Commercial Sampling and Analysis of Pig Iron," p. 19. U. S. Steel Corporation (1912).

<sup>28</sup> H. E. Walters, *Chem. News* 84, 239 (1901).

<sup>28a</sup> S. N. Rozanov and D. V. Voskresenskaya, *Z. Pflanzenernähr., Düngung Bodenb.* A35, 140-6 (1934).

<sup>28b</sup> V. G. Funk, *Zavodskaya Lab.* 3, 698-9 (1934).

<sup>29</sup> The Chemists' Committee of the U. S. Steel Corp., "Methods for the Commercial Sampling and Analysis of Plain Steels," p. 18. U. S. Steel Corporation (1914).

<sup>30</sup> L. Dufty, *Chem. News* 84, 248 (1901).

<sup>31</sup> The Chemists' Committee of the U. S. Steel Corp., "Methods for the Commercial Sampling and Analysis of Alloy Steels," p. 26. U. S. Steel Corporation (1915).

and mix. Decant through a dry filter, transfer 50 cc. to a beaker, add 10 cc. of concentrated nitric acid, boil and cool. This corresponds to a sample of 0.2 gram. The method is applicable in the presence of tungsten.

*Minerals, Rocks and Soil.* Grind the rock or soil fine enough to pass through a 150 mesh sieve.<sup>8</sup> Mix 1 gram of sample with 4 grams of dry manganese-free sodium carbonate and fuse in a platinum crucible until the melt is clear, usually about 10 minutes. Pour the hot fusion into a platinum dish and rotate so as to form a thin film. Put the crucible in the dish with the fusion. Add about 100 cc. of water and heat on the water bath until the product is disintegrated. Remove the crucible and wash. Acidify the mixture with 130 cc. of 1:5 sulfuric acid and dilute to 250 cc. If a precipitate of silica remains, filter through a dry filter, preferably on a Büchner funnel. The silica usually produces only an opalescence. The color may be estimated in the presence of this opalescence if it is slight.

Using direct extraction of the sample with sulfuric and hydrofluoric acid Gortner and Rost found variations as great as 200 per cent. From 3 to 4 extractions, each taking about 3 hours, are required to obtain results having any value. To avoid formation of a precipitate of silicic acid dissolve the melt, after fusion, in water. Pour the aqueous solution of the melt into 130 cc. of 1:5 sulfuric acid. A colloidal solution of silicic acid is obtained which will keep for weeks or which can be boiled without precipitation occurring.<sup>33</sup>

If the sample contains chromium the yellow color of chromate ion will modify the color of the final solution. To separate manganese from chromium<sup>34</sup> render the acid solution of the fusion just alkaline with ammonium hydroxide and warm for 5 minutes. This precipitates the manganese and iron as hydroxides. Filter and wash thoroughly. Dissolve the manganese on the filter in 1:5 sulfuric acid with the addition of sulfurous acid or hydrogen peroxide. Heat to decompose excess sulfurous acid or hydrogen peroxide. The filtrate may be used for determination of chromium.

*Water.*<sup>35</sup> To 100 cc. of water or a sample containing less than 1 mg. of manganese add 10 cc. of 1:3 nitric acid and 1 cc. of concentrated sulfuric acid. Evaporate until most of the sulfuric acid is driven off. This destroys organic matter and drives off chlorides. By not taking to dryness the manganese sulfate is easily soluble. Only a small amount of sulfuric

<sup>33</sup> C. Newcomb, *Analyst* 53, 644-5 (1928).

<sup>34</sup> M. Dittrich, *Z. anorg. Chem.* 80, 171 (1913).

<sup>35</sup> W. D. Collins and M. D. Foster, *Ind. Eng. Chem.* 16, 586 (1924).

acid should be left as it the

air been

to remove oxides of nitrogen.

A double oxidation with

or single oxidation

American Public Health Asso-

cc. of the cyanide

10

an efficient hood. Boil until all

cyanide is expelled, cool and dilut

The method was originally de

by taking the other metals known to be present into

other

oxidizing agents may be used.

*Phosphoric Acid.*<sup>39</sup> Dilute 5-10 grams of the 20 cc. of concentrated nitric acid and heat to boiling. Add sufficient 1 per cent silver nitrate solution to precipitate all chlorides, and 3 cc. in excess. Boil to coagulate the precipitate, cool and dilute to a definite volume. Use t of the supernatant liquid as sample.

*Pharmaceutical Preparations.* This method :

preparations such as peptonates, syrup

thoroughly

on the filter with the solution

to expel hydrogen sulfide and add 1 cc. of 1:3 nitric acid and 1 drop of 2 per cent silver nitrate solution. If this causes turbidity indicating imperfect washing of sulfides add a very slight excess of 2 per cent silver nitrate solution, boil again and filter.

The method is applicable in the presence of 3-5 times as much iron as manganese, with persulfate oxidation.

of

6th ed., pp. 50-1 (1925)

<sup>38</sup> J. E. Clennell, *Eng. Mining J.* 78,

<sup>39</sup> W. H. Ross, C. B. Durgin and R.

<sup>40</sup> M. R. Schmidt, *J. Am. Chem. Soc.* 32, 965 (1910).

*Blood, Tissue or Vegetable Matter.*<sup>41</sup> To the sample of 10–100 grams add 25 cc. of concentrated sulfuric acid and heat to boiling for 5 minutes. This separates water from fat. When cool add 30 cc. of concentrated nitric acid and 10 cc. of concentrated hydrochloric acid. Let stand over night. Separate fat which has risen to the surface and boil off the excess acid to give a few cc. of clear liquid containing the manganese as sulfate.

*Blood, Tissue or Vegetable Matter—Alternative Method.*<sup>41</sup>—Free animal tissue from adhering fat or connective tissue and rinse with distilled water. Heat the sample of 10–25 grams of blood,<sup>43</sup> animal tissue or vegetable matter<sup>45</sup> on a sand bath in a quartz beaker until entirely dry, without allowing it to fuse. Carefully heat the upper part of the beaker with a burner to volatilize or dehydrate tarry matter. Blanks must be run on the quartz beakers used, to insure absence of manganese.

Heat in an electric furnace at 600–700° until the carbon has nearly disappeared. Add 2 to 4 grams of a mixture of equal weights of sodium and potassium nitrates, 10 cc. of concentrated sulfuric acid and 5 cc. of concentrated hydrochloric acid. At this stage much of the manganese is present as the sesquioxide which is difficultly soluble in nitric acid but easily soluble in hydrochloric acid. Warm gently, evaporate to dryness, then heat to a cherry red. A few cc. of a clear fusion should be obtained. If a large quantity of carbon is present, when cool add 5 cc. of concentrated nitric acid. Add 1 cc. of concentrated sulfuric acid and heat until the fusion is red. Rotate so as to form a thin layer or the melt will expand in cooling and may crack the beaker. If the melt hardens at a high temperature, let cool for 2 minutes, add 5 cc. of concentrated sulfuric acid and heat as before. After the melt has hardened and cooled add 50 cc. of water and heat on a sand bath until dissolved. Filter off silica or carbon. If silica remains in the filtrate add 1 cc. of hydrofluoric acid and warm. Add 1 cc. of concentrated nitric acid and use for oxidation.

If more than 2–3 mg. of manganese are present manganese dioxide may precipitate on oxidation. In that case dissolve the precipitate by addition of saturated sulfur dioxide solution, dilute to a standard volume and take an aliquot for development of color. If the salt concentration in the final solution is too great the manganese color will not develop. In that case dilute until estimation is possible. By such treatment of

<sup>41</sup> C. K. Reimann and A. S. Minot, *J. Biol. Chem.* 42, 329-46 (1920).

<sup>43</sup> G. Bertrand and F. Medigreceanu, *Ann. de l'inst. Pasteur* 26, 1013-29 (1912); *Ibid.* 27, 1-11 (1913).

<sup>45</sup> G. Bertrand and M. Rosenblatt, *Ibid.* 35, 815-9 (1921).

samples the general occurrence of manganese in the animal and vegetable kingdom has been demonstrated.

*Textiles.*<sup>46</sup> Follow the preparation of sample as for copper<sup>47</sup> until rendered just acid to Congo red. Add 5 cc. of 10 per cent sulfuric acid and dilute to 200 cc. As a solution for preparation of standards, add 2.5 grams of potassium sulfate, 2.5 grams of sodium sulfate and 10 grams of ammonium sulfate to water. Dissolve, add 5 cc. of 10 per cent sulfuric acid and dilute to 200 cc. Use this in parallel with the sample.

*Urine.*<sup>48</sup> To 100 cc. of urine in a Kjeldahl flask, or more, if the quantity of manganese is less than 1 p.p.m., add 20 cc. of concentrated nitric acid. Evaporate to a paste on a sand bath. Let cool, add 5 cc. of concentrated sulfuric acid and heat until about 1/3 of this is driven off as sulfur trioxide. More sulfuric acid may be needed if phosphates are high or the quantity of sample is large. Cool, add 5 cc. of concentrated nitric acid and heat until the brown fumes disappear. Repeat until oxidation is complete. Add distilled water and 5 cc. of concentrated nitric acid, and dilute to 100 cc. If necessary filter off silica resulting from the attack on the glassware by phosphoric acid.

The amount of manganese in one normal urine is reported as less than 0.02 p.p.m. The amount of sample which can be taken is limited to about 2 liters because of the concentration of salts built up.

**Procedure—Oxidation in Acid Solution—Lead Peroxide.**<sup>4,50</sup> To 10 cc. of sample in a tube add 3 cc. of 1:1 nitric acid and place a funnel in the neck. Heat on a calcium chloride bath boiling at 115°. When the solution begins to boil add 0.5 gram of fine lead peroxide. Boil gently for 5 minutes and remove. Let cool and settle in a dark place. Decant into comparison tubes and compare by dilution with a standard similarly treated.

The lead peroxide must show no color on heating 5 minutes with 1:1 nitric acid. It may be prepared by boiling red lead with 1:3 nitric acid, decanting the upper layer of lead nitrate solution, filtering and washing with hot water.

*Persulfate.* To the sample in acid solution add 1 cc. of 2 per cent silver

---

<sup>46</sup> Wm. C. Smith, *Proc. Am. Assoc. Textile Chem. Colorists* 1930, 217-9; *Am. Dyestuff Repr.* 19, 583-5 (1930).

<sup>47</sup> See p. 155.

<sup>48</sup> R. F. McCrackan and E. Passamaneck, *Arch. Path. Lab. Med.* 1, 586 (1926).

<sup>50</sup> G. Lunge, "Technical Methods of Chemical Analysis," vol. 2, p. 65. D. Van Nostrand Co., New York (1911).

nitrate solution and 1 gram of moist potassium or ammonium persulfate per milligram of manganese. Heat in a water bath until the permanganate color is completely developed, usually about 10 minutes. Cool, transfer to a comparison tube, dilute to 50 or 100 cc. and match the color against a standard containing the same amounts of acid and salts. The silver nitrate acts as a catalyst and in its absence oxidation may be incomplete.<sup>5</sup> If excess persulfate is present the color remains unchanged for several days. It may be allowed to stand over night for particles producing turbidity to settle.<sup>41</sup> Loss of manganese in filtration to remove turbidity may be avoided by using an excess of the oxidizing agent.<sup>53</sup>

For examination of vegetable ash, Wester<sup>53</sup> adopted potassium persulfate as oxidizing agent and parallel oxidation of reduced manganese for development of the standard color. He found the color did not vary with the use of between 2.5 and 10 per cent of 1:1 nitric acid. With less it was darker and with more it was lighter. No effect on the color of 10 cc. was caused by 1–10 drops of concentrated sulfuric acid or 10 per cent silver nitrate, by 0.25–1.0 gram of persulfate or by heating from 15 to 30 minutes. Over 0.2 mg. of manganese per 10 cc. gave turbidity. The presence of excess chloride must be avoided. Iron causes the color to tend toward brown.

If the solution for estimation by the persulfate method has been acidified with phosphoric acid instead of sulfuric acid, there will be less interference by chlorides.<sup>55</sup>

*Sodium bismuthate.* To the sample in acid solution add 0.1 gram of sodium bismuthate per milligram of manganese. Boil if necessary, to obtain the true permanganate color. Stir and let settle. Filter through a Gooch crucible containing a mat of ignited asbestos which has been washed with permanganate solution and then with distilled water. Dilute to 50 or 100 cc. and compare with a standard permanganate solution containing the same kind and quantity of acid and salts as the sample.

As an alternative procedure let excess bismuthate settle in the dark for 30 minutes and pipet a portion of the clear upper layer for comparison.<sup>30</sup>

*Periodate.*<sup>7</sup> Adjust the concentration of acid so that 100 cc. of solution contains at least 5 to 6 cc. of concentrated sulfuric acid, 20 cc. of concentrated nitric acid, 5 to 10 cc. of syrupy phosphoric acid, or a

<sup>53</sup> A. C. Janzig, *J. Am. Water Works Assoc.* 18, 744-5 (1927).

<sup>54</sup> D. H. Wester, *Rev. trav. chim.* 39, 414-22 (1920).

<sup>55</sup> F. Alten and H. Weiland, *Z. Pflanzenernähr., Düngung Bodenk.* 30A, 193-8 (1933).

corresponding mixture of acids. In the presence of strong reducing agents use nitric acid and boil, adding a little persulfate if carbon compounds are present. If the sample contains chlorides evaporate with nitric and sulfuric acids to fumes of sulfur trioxide, taking up with an acid solution as above. Add 0.2 to 0.4 gram of potassium or sodium periodate and boil one minute. If the double salt,  $\text{Na}_3\text{H}_2\text{IO}_6$ , is used take about 1.5 times as much. Keep hot 5 to 10 minutes, let cool and dilute to 100 cc. Compare the color with that of a standard solution subjected to identical treatment.

The high concentration of acid is necessary to prevent precipitation of manganese as well as other metals such as silver, lead, mercury or bismuth, as iodate or periodate. A higher concentration of acid than given does no harm. Iron, if present in considerable amounts, is kept in solution by sulfuric or phosphoric acid. Phosphoric acid is added to remove the color produced by iron, or a known amount of ferric nitrate solution is added to the standard to equalize such color in the sample. About 1 per cent error is reported with this method.

For routine work a standard solution containing a slight excess of periodate, kept stoppered, will be stable for at least 3 months. If excess periodate is present the standards are also stable for several months. More than a trace of chlorides must be absent from the sample.

*Oxidation in Alkaline Solution.* This has been recommended over oxidation in acid solution when much iron is present because of the interference of the yellow color of iron with the purple of permanganate.

*Hypochlorites.*<sup>59</sup> To the sample add 1 drop of 10 per cent copper sulfate solution. Treat with excess of a moderately alkaline hypochlorite solution. Copper oxide is precipitated and the supernatant liquid may be used for quantitative comparison. For convenience use a 50 cc. sample, dilute the solution after oxidation to 100 cc., allow to settle and pipet 50 cc. of the upper layer for examination.

*Hydrogen Peroxide.*<sup>59</sup> Treat the acid solution of the sample with excess of powdered zinc oxide. Dilute to a definite volume and filter through a dry filter paper, discarding the first 10 cc. All trivalent metals and copper remain in the precipitate. Add an aliquot of the filtrate drop by drop with stirring to 10 cc. of a 3 per cent hydrogen peroxide solution containing excess of 10 per cent potassium hydroxide. Compare with the color of a standard manganous sulfate solution similarly treated. The method detects 0.02 mg. of manganese.

Lead does not interfere. If nickel or cobalt are present add potassium

---

<sup>59</sup> J. Heslinga, *Chem. Weekblad* 19, 302-3 (1922).



## BY *o*-TOLIDINE

cyanide to the peroxide solution and be careful to maintain alkalinity at all times.

*Air or Oxygen.*<sup>18</sup> To 5 cc. of sample add 3 cc. of glycerol and 1 cc. of approximately 50 per cent sodium hydroxide solution. Blow a slow current of air through the cold solution for 20 minutes, or of oxygen for 10 minutes.

The method is based on the solubility of manganous hydroxide in glycerol and the ease with which it is oxidized by air to permanganate. If insoluble hydroxides are precipitated by alkali allow to settle and test a clear aliquot of the upper layer.

**Standards—Permanganate.** Dissolve 0.2877 gram of pure potassium permanganate in water and dilute to 1 liter. Each cc. corresponds to 0.1 mg. of manganese. Collins and Foster<sup>35</sup> use a standard permanganate solution directly in the final comparison and state that there is no advantage in reducing it first in order to give it the same treatment as the sample.

**Manganous Sulfate.** Other investigators recommend the reduction, by dissolving the potassium permanganate in 20 per cent sulfuric acid and cautiously decolorizing with dilute sulfurous acid,<sup>8</sup> or by acidifying an aqueous solution of potassium permanganate with sulfuric acid, heating to boiling, and slowly adding enough dilute oxalic acid solution<sup>63</sup> to discharge the color before diluting to volume. This results in a standard manganous sulfate solution which is given the same treatment as the solution of sample.

**Iron or Steel.** As a standard select a similar iron or steel containing 0.4 to 0.5 per cent of manganese. Treat 0.2 gram of this exactly the same as the sample. Add the same oxidizing agent and compare with the sample.

## MANGANESE BY *o*-TOLIDINE

Manganese in the absence of free chlorine can be determined with *o*-tolidine.<sup>64</sup> The color developed is the same yellow as for free chlorine and the same standards are used. The color is obtained with manganic compounds but not with manganous so that mild preliminary oxidation in alkaline solution is necessary.

<sup>63</sup> American Public Health Association, "Standard Methods of Water Analysis," 6th ed., p. 50 (1925).

<sup>64</sup> Leroy Forman, *J. Am. Water Works Assoc.* 21, 1212-7 (1929).

## MANGANESE

e—To 100 cc. of  
sodium hydroxide solution  
oxide  
note

. Bubble oxygen through  
30 minutes. If mor  
phosphoric acid.

5 cc. of the *o*-tolidine  
e after  
multi  
angan

the permanent

basis  
with  
has been reported with tetrame

same weight of manganese-irre  
sample is of iron or steel. An equiv  
be added instead of this iron wire.  
acid and evaporate to dryness. Heat to  
residue in a minimum volume of 1 :  
1 : 1 sulfuric acid and evaporate to  
oration is simply a convenient

<sup>65</sup> See p. 538.  
ee p. 540.  
talph G. H

200 cc. and filter into a 250 cc. flask. Dilute to volume with distilled water.

**Procedure**—Place 1 cc. of sample and 1 cc. of standard manganese solution in 10 cc. flasks. Dilute with distilled water to about 6 cc. Add 1 cc. of a 0.5 per cent solution of tetramethyldiaminodiphenylmethane and 1 cc. of glacial acetic acid. Mix and add 0.5 cc. of *N* sodium hydroxide solution. Mix and add glacial acetic acid, drop by drop, until the maximum color is developed. Dilute to 10 cc. and compare.

If the color developed is too deep or if a precipitate is formed, dilute the sample and standard further and repeat the development of color.

**Standards**—Dissolve 0.158 gram of pure manganese dioxide in 10 cc. of concentrated hydrochloric acid and dilute to 1 liter. Each cc. contains 0.1 mg. of manganese. The standards fade slowly. The color shades can be matched with mixtures of 0.01 per cent solutions of crystal violet and methylene blue.

#### MANGANESE BY BENZIDINE

Manganese in the form of manganate ion reacts with benzidine hydrochloride in dilute solution to produce a brilliant blue-green color.<sup>69</sup> The color is also given by other oxidizing agents such as titanium, cerium, chlorine, etc. The reaction, sensitive to 8 parts per billion, is believed to be due to an autoxidation. It has been applied to micro technique<sup>70</sup> and to macro estimation of manganese,<sup>71,72</sup> or conversely to chlorine in the absence of manganese.<sup>73</sup>

The reaction is affected by carbonates or organic matter, the quantity of benzidine used, and pH. The color produced is much more intense than that of permanganate ion or the product of its reaction with *o*-tolidine. The color fades in about 2 minutes to an unstable greenish yellow. The comparison is made, therefore, either with a fresh series of standards or, more conveniently, with artificial standards. Large amounts of sulfate ion interfere because of the formation of insoluble benzidine sulfate.

<sup>69</sup> Feigl, *Chem.-Ztg.* 44, 689-90 (1920).

<sup>70</sup> Feigl, *Microchemie* 1, 74-8 (1923).

<sup>71</sup> Olszewski, *Chem.-Ztg.* 47, 273-4 (1923).

<sup>72</sup> R. C. Stratton, J. B. Ficklen and W. A. Hough, *Ind. Eng. Chem., Anal. Ed.* 4, 2 (1932).

<sup>73</sup> Olszewski, *Chem.-Ztg.* 47, 649-50 (1923); *Pharm. Zentralhalle* 68, 733-5 (1927).

The speed of fading of the color difficult.<sup>13</sup> Sensitivity and salts by have been investigated.

to contain 0.0001 to 0.001

**Standard**—This is prepared empirically to match a solution containing 0.0003 mg. of manganate ion per 100 cc. To a 15 per cent solution of copper sulfate add a 0.5 per cent solution of picric acid until the correct tint is obtained. Then dilute until it matches the desired standard. It can then be used for balancing over the desired range.

## CHAPTER XXX

### ZINC

#### ZINC BY RESORCINOL

ZINC reacts with resorcinol in alkaline solution to produce a blue color.<sup>1</sup> The character of this alters on exposure to the air. The addition of hydrochloric acid turns the solution red and this color is not altered by exposure. It has been shown that in the absence of air the blue compound is not formed in alkaline solutions containing ammonium hydroxide.<sup>2</sup> In the absence of ammonia a green color is developed at pH 9.2 and a red one on the acid side. Addition of ammonia changes this irreversibly into the blue and red compounds discussed in the procedure. Over the range pH 4.3-5.9 the color is changing and is violet. Both colors can be extracted with amyl alcohol.

Doubt as to the reliability of the method in alkaline solution has been raised by separation of crystals of resorcinol from anhydrous ether which gave the same blue on exposure to air. Zinc catalyzes the development of this blue color.<sup>3</sup> Calcium, nickel and cobalt must be absent.

A sample solution suitable for examination by this method must contain between 0.0001 mg. and 0.2 mg. of zinc per cc. By exercising care accuracy to 6 per cent is obtained.

**Procedure**—Dissolve the sample in 1:2 nitric acid, dilute to 75 cc. and neutralize with 1:1 ammonium hydroxide. Dilute to 96 cc. Add 2 cc. of concentrated ammonium hydroxide and 2 cc. of a 5 per cent solution of resorcinol in alcohol. The color develops at once.

After results have been obtained in alkaline solution add a slight excess of hydrochloric acid and check the first result by comparing the red color produced in acid solution. The concentration curve by the balancing method varies widely from Beer's law.

<sup>1</sup> Angel del Campoy Cerdan and J. de la Puente, *Anales Soc. espan. fis. quim.* 11, 98-108 (1913).

<sup>2</sup> Ligor Rey and M. Faillebin, *Compt. rend.* 188, 1679-88 (1929).

<sup>3</sup> Herm. Mohler and Rose Widmer, *Mitt. Lebensm. Hyg.* 22, 130-3 (1931).

**Standard**—Dissolve 0.1000 gram of pure zinc in 10 cc. of 1:1 nitric acid and dilute to 1 liter. Treat a suitable amount of this in the same way as the sample. The color of the standard must be very close to that of the sample as the dilution method does not hold through any considerable range. Alteration due to exposure to air can be prevented by covering sample and standards with a thin layer of mineral oil. For the duplication method the blank should contain 70 cc. of water, 2 cc. of concentrated ammonium hydroxide and 2 cc. of 5 per cent resorcinol solution. An error may be introduced due to variation in the dissolved salts.

#### ZINC AS THE SULFIDE

Zinc may be estimated turbidimetrically<sup>4</sup> or nephelometrically<sup>5</sup> as the white, flocculent sulfide, if present in a concentration of 0.05 per cent or less. If less than 0.5 mg. of zinc per liter is present, the accuracy of estimation is to about 0.1 mg.

**Sample—Tissue.**<sup>5</sup> Add 3 cc. of concentrated sulfuric acid to a 2 gram sample in a 50 cc. Kjeldahl flask. Add concentrated nitric acid, drop by drop, as long as reaction occurs. Let stand over night, add 1 cc. of 30 per cent hydrogen peroxide, and heat cautiously. If necessary, let cool, and add more hydrogen peroxide until the solution is colorless. Transfer the solution quantitatively to a quartz dish and evaporate to dryness to remove all excess acid. Dissolve the residue in 5 cc. of 1:1 hydrochloric acid and transfer to a 25 cc. centrifuge tube. Dilute with the washings to 25 cc. Centrifuge and wash the residue with 5 cc. portions of 1:1 hydrochloric acid and two 5 cc. portions of water. Stir up the residue with the wash solution each time. Collect the solution and washings in a 50 cc. Erlenmeyer flask for use in the procedure.

**Iron and Steel.** The primary problem in this case is removal of iron. It is successfully removed<sup>7</sup> by precipitation with ammonium hydroxide,<sup>8</sup> by ether extraction<sup>9</sup> or by precipitation of the zinc with hydrogen sulfide<sup>10</sup> in solution in hydrochloric acid adjusted to the optimum pH with

<sup>4</sup> L. W. Winkler, *Z. angew. Chem.* 26, 38 (1913).

<sup>5</sup> Ludwig Pincussen and Ernst Brück, *Biochem. Z.* 265, 58-60 (1933).

<sup>7</sup> H. A. Bright, *Bureau of Standards J. of Research*, 12, 383-9 (1934).

<sup>8</sup> W. D. Collins, *et. al.*, *Ind. Eng. Chem., Anal. Ed.* 4, 154-6 (1932).

<sup>9</sup> G. E. F. Lundell, J. I. Hoffman and H. A. Bright, "Chemical Analysis of Iron and Steel," p. 387. John Wiley and Sons, New York (1931).

<sup>10</sup> F. G. Hills, *Ind. Eng. Chem., Anal. Ed.* 5, 201 (1933).

citric acid and sodium citrate. Only the ether method will be given, as related to the procedure which follows.

Transfer 25 grams of sample to a Pyrex beaker and dissolve by gentle heating with 150 cc. of concentrated hydrochloric acid and 50 cc. of water. Cool and dilute to 250 cc. Filter and wash the paper with water. Add 30 cc. of 1:1 nitric acid to oxidize the iron and gradually heat to boiling. If the reaction becomes too vigorous, cool with water. Evaporate to 50 cc. and add 30 cc. of concentrated hydrochloric acid. Again evaporate to about 50 cc. Add 250 cc. of 1:4 hydrochloric acid and cool to room temperature.

Transfer to a separatory funnel and cool to 5°. Add 550 cc. of ethyl ether and shake for a few minutes. Allow to separate for 10 minutes and draw off the aqueous layer into the original beaker. Discard the ether and return the acid solution to the separatory funnel with 200 cc. of fresh ether. Shake and separate as before. Warm the acid solution until free from ether and concentrate to 50 cc. Add 14 cc. of 1:1 sulfuric acid and 15 cc. of concentrated nitric acid. Evaporate to sulfur trioxide fumes and continue the heating without decomposing the salts until as much as possible of the excess acid is removed.

Cool, add 100 cc. of water and heat until solution is complete. Pour the solution at about 75° into 100 cc. of 1:1 ammonium hydroxide containing 5 grams of ammonium sulfate. Filter and wash the precipitate with water. Add hydrochloric acid to the filtrate until slightly acid and concentrate to a suitable volume. Use this or an aliquot for the procedure.

*Other Samples.* The final solution should be in hydrochloric acid and may contain moderate amounts of iron, manganese, copper and lead.

**Procedure**—Neutralize or acidify the sample solution to about 0.1 *N*. Heat it on the water bath and pass in hydrogen sulfide until an excess is present. Stopper and let stand over night for precipitation of sulfides of copper and lead. Addition of a couple of cc. of ether facilitates this precipitation. Filter through a very small, hardened paper. Boil the filtrate until free from hydrogen sulfide. Concentrate to about 20 cc., or dilute to a known volume and take a 20 cc. aliquot.

To remove iron, add 6 drops of concentrated nitric acid and transfer to a 25 cc. centrifuge tube. Add 30 per cent sodium hydroxide solution until distinctly alkaline. Acidify by careful addition of glacial acetic acid, drop by drop. Centrifuge to separate the precipitate. Dissolve the residue in 2 cc. of 1:1 hydrochloric acid and repeat the treatment with sodium hydroxide and glacial acetic acid until the precipitate is red-

brown. Combine the sample solution and washings in a 50 cc. flask. Heat nearly to boiling, saturate with hydrogen sulfide, stopper and let stand over night. Filter the precipitated zinc sulfide on a small, hardened filter paper. Wash the filter with 1:100 acetic acid, dry and ash. Transfer the ash to a centrifuge tube with 2 cc. of glacial acetic acid and dilute to 5 cc. Centrifuge and decant. Wash twice by stirring with 5 cc. of 1:100 acetic acid. Combine the wash solutions in a 25 cc. Nessler tube. In a similar tube take a suitable volume of a standard zinc solution and 2 cc. of glacial acetic acid. Dilute to about 15 cc. with 1:100 acetic acid.

To sample and standard add 5 cc. of a saturated solution of hydrogen sulfide and 1 cc. of *N* hydrochloric acid. Mix well and add 2 cc. of 15 per cent ammonium acetate solution. Dilute to 25 cc. and compare nephelometrically or turbidimetrically.

The duplication method may also be used for preparation of the standards, or a series of temporary standards may be prepared.

**Standard Solution**—Dissolve 0.5000 gram of pure zinc in 500 cc. of 0.1 *N* hydrochloric acid. Each cc. contains 1 mg. of zinc.

#### ZINC BY POTASSIUM FERROCYANIDE

The well known potassium ferrocyanide precipitation of zinc can be adapted to nephelometric estimation by careful control of acidity, salt concentration and time of standing.<sup>11</sup> Iron and copper must be removed. Analyses of 33 samples containing 0.50–1.40 mg. of zinc showed an average error of  $\pm 0.06$  mg. By a somewhat different procedure accuracy to 0.05 mg. has been obtained on food products<sup>12</sup> and 1 p.p.m. in water analysis.<sup>13</sup>

**Sample**—*Organic Materials, Only Traces of Arsenic and Lead Present.*<sup>13a</sup> Ash 1–5 grams in a muffle at a low red heat, avoiding fusion of the ash. Zinc is not volatilized under these conditions. Add 15 cc. of water and 7 cc. of 3 *N* hydrochloric acid to the ash. Heat on the steam bath until half the liquid has evaporated. Further evaporation may cause gelatinous silica to separate. If trouble is encountered dehydrate

<sup>11</sup> Lawrence T. Fairhall and John R. Richardson, *J. Am. Chem. Soc.* 52, 938–44 (1930).

<sup>12</sup> V. Birekner, *J. Biol. Chem.* 38, 191 (1919).

R. Meldrum, *Chem. News* 116, 271, 295, 308 (1917).

<sup>13</sup> P. L. Howard, *Ind. Eng. Chem., Anal. Ed.* 6, 423–5 (1934).



the silica at  $110^{\circ}$  as usual, redissolve in *N* hydrochloric acid and proceed. Filter and wash the filter until the filtrate is about 25 cc. If much unburned carbon remains on the filter, ignite again, extract and join the extract with the previous filtrate. The insoluble ash normally contains little zinc.

Add a few drops of bromophenol blue solution and add 1:1 ammonium hydroxide until the solution is blue. Add *N* hydrochloric acid until the solution just turns yellow and precipitated iron and aluminum are redissolved. Prepare a citrate buffer by dissolving 12 grams of sodium citrate and 23 grams of citric acid in water, dilute to 100 cc. and adjust to pH 3.<sup>13aa</sup> Add 3–5 cc. of this buffer according to the iron content to retain the iron in solution. Excess buffer does no harm. Heat on the steam bath and add 1:10 hydrochloric acid or 1:10 ammonium hydroxide until the color is grey, which is a pH of 3.0–3.4. If iron interferes with reading the indicator, reduce with sulfur dioxide or sodium sulfite. Pass hydrogen sulfide through the solution for 1–2 minutes, saturating it. If there is not much precipitate of lead or copper add 1 cc. of a 5 per cent solution of acid-washed talc. Mix, set in cold water, and pass hydrogen sulfide through the solution until it is cold. Filter at once or stopper until ready to filter.

Filter on a close textured paper and wash with 0.045 per cent formic acid saturated with hydrogen sulfide. To remove all iron, at least 5–6 washings are required. The residue on the paper is zinc sulfide with arsenic, copper or lead sulfide. Not more than 1–2 mg. of arsenic nor 10 mg. of lead should be present.

Dissolve the zinc sulfide from the filter with 15 cc. of *N* hydrochloric acid in 2–3 cc. portions and collect in a 20 cc. flat-bottomed tube. Some of the lead sulfide is also dissolved. Add 2 cc. of 5 *N* sodium hydroxide solution and dilute to 20 cc. with *N* hydrochloric acid.

*Organic Matter, much Arsenic or Lead Present.* Ash and dissolve the ash as for the preceding method. Render the solution 6 *N* with hydrochloric acid and saturate with hydrogen sulfide. Stopper the flask lightly and warm on the steam bath. Filter the arsenic sulfide. Evaporate in an evaporating dish nearly to dryness. Transfer to a small flask with water and add hydrochloric acid to 0.25 *N*, pH 0.52. Saturate with hydrogen sulfide, stopper lightly, and warm on the steam bath. Filter the incompletely precipitated lead sulfide and neutralize the filtrate to pH 3 as in the previous method. Add buffer and proceed as for low arsenic and lead contents.

<sup>13aa</sup> See p. 696 et seq. for estimation of pH.

*Organic Materials. Alternative Method.* Ash 2 grams in a muffle at a low red heat, avoiding fusion of the ash. Extract the ash with 3 cc. of 3 *N* hydrochloric acid and 10 cc. of water. Filter and evaporate to half volume. Add 10 cc. of 10 per cent oxalic acid solution and continue to evaporate to about 10 cc. If copper is present saturate with hydrogen sulfide and let stand over night. Filter or centrifuge to remove copper sulfide and oxalates of lead and calcium. Wash the precipitate with 5 per cent oxalic acid solution. To the clear filtrate add 10 cc. of 10 per cent oxalic acid solution and 1 cc. of 4 per cent potassium ferrocyanide solution. Mix and after 15 minutes filter the zinc ferrocyanide, if necessary adding acid-washed tale as a filter aid. Wash the filter with 5 per cent oxalic acid solution. As used this solution must be more than 0.5 *N* to prevent redissolving of lead and calcium oxalates and about two-thirds saturated with oxalic acid to prevent development of blue due to iron. The method is simpler than the preceding one when much arsenic or lead is present.

Decompose the precipitate on the filter by washing with 10 cc. of *N* sodium sulfide solution. Wash 3 times with 0.25 *N* sodium sulfide solution. Wash once with water. Dissolve the zinc from the filter with 15 cc. of *N* hydrochloric acid in 2-3 cc. portions. Collect in a 20 cc. flat-bottomed tube and add 2 cc. of 5 *N* sodium hydroxide solution and dilute to 20 cc. with *N* hydrochloric acid.

**Procedure**—Prepare a series of standards containing 0.02, 0.04, 0.06, 0.08 and 0.10 mg. of zinc. Dilute each of these to 15 cc. with *N* hydrochloric acid, add 2 cc. of 5 *N* sodium hydroxide solution and dilute to 20 cc. with *N* hydrochloric acid.

To the sample and to each standard add 2 drops of 2 per cent potassium ferrocyanide solution and mix at once. Let stand for 15 minutes and compare against a dead black surface. Comparison may also be made in Nessler tubes. In that case paint the bottoms black and paint a black line around the meniscus.

**Standard**—Prepare a standard zinc solution by dissolving 0.1000 gram of pure zinc in *N* hydrochloric acid and dilute to 1 liter with the same acid. Each cc. of this contains 0.1 mg. of zinc. For use dilute 10 cc. of this stock solution to 100 cc. Each cc. now contains 0.01 mg. of zinc.

#### ZINC BY DIPHENYLTHIOCARBAZONE (DITHIZONE)

Zinc in alkaline solution gives a red color with dithizone in carbon

tetrachloride.<sup>13b</sup> The color migrates into the aqueous phase more or less according to the alkalinity. The excess of dithizone affects the color. Copper interferes. From 1–0.001 mg. of zinc can be estimated with accuracy to about 7 per cent.

**Reagent**—Saturate 100 cc. of 0.01 *N* sodium hydroxide solution by shaking for 3 minutes with 0.5 gram of dithizone. Filter and use as long as it is clear. Turbidity normally appears in 1–2 days. The color is about that of 0.1 per cent methyl orange solution. One cc. of reagent is sufficient for about 0.003 mg. of zinc.

**Sample**—*Urine or Feces*. Ash 10–100 cc. of urine or 1–10 grams of feces with 1–10 cc. of concentrated sulfuric acid and a few cc. of concentrated nitric acid. Evaporate to sulfur trioxide fumes to drive off all nitric acid. Dilute the cooled residue with 10 volumes of water. Add 2 drops of 10 per cent copper sulfate solution and pass in hydrogen sulfide for 20 minutes. This removes copper originally present as well as that added. Filter at once, wash the filter and evaporate to dryness. Heat until the excess of sulfuric acid is substantially all driven off. Dissolve the residue in water and dilute to 20 cc. Titrate 5 cc. with 0.01 *N* sodium hydroxide solution to determine the acidity. Exactly neutralize the remaining volume of sample without adding indicator and dilute to a known volume according to the general preparation of samples below.

The sample can also be ashed in a muffle<sup>13c</sup> below 450° and the ash dissolved in acid and copper precipitated as above.

**General**. The alkalinity or acidity of the sample used must be known in order to produce the same amount in the standard. A preliminary estimate of the zinc concentration is necessary to make the proper dilution.

Neutralize the sample with *N* sodium hydroxide or *N* hydrochloric acid on the basis of titration of an aliquot, and dilute to a definite extent. Pipet 1 drop of reagent and 1 drop of sample into a porcelain dish. An immediate cherry red precipitate indicates more than 0.01 mg. of zinc per drop. Slow appearance of a red color indicates 0.001–0.01 mg. of zinc per drop. A pale red indicates less than 0.001 mg. of zinc per drop. If necessary dilute the sample further so that 1 cc. contains 0.001–0.003 mg. of zinc.

<sup>13b</sup> W. Deckert, *Z. anal. Chem.* 100, 386-90 (1935).

<sup>13c</sup> Ph. K. Thompson, *J. Ind. Hygiene* 7, 358 (1925).

time.

of zinc. 1, 0.002 or 0.003 mg.

3. Add *N* sodium

#### ZINC BY PHOSPHOMOLYBDOTUNGSTIC ACID

IS THEN REQUIRED.

Aluminum and bismuth must be absent.

**Procedure**—Follow that given for aluminum.<sup>15</sup>

#### ZINC BY UROBILIN

Zinc gives a reaction with urobilin or stercobilin to form a green fluorescent substance in faintly alkaline alcoholic solution.<sup>13a</sup> The color from 0.001 mg. can be detected with a strong arc light and an ultraviolet filter. Above 0.01 mg. the fluorescence is too strong for estimation. In the range of 0.001–0.01 mg. the accuracy is to about 10 per cent.

<sup>1</sup>, *Z. anal.* .. 82, 366-74 (1930).

<sup>13</sup>, 273-90 (1925).

The alcohol used must be highly purified. Copper, calcium, nickel, cobalt, magnesium, sodium and potassium interfere if present in an amount equal to that of the zinc. Cadmium increases the color. Because of the nature of the color developed only the series of standards method is applicable.

**Sample—Organic matter.** Dry and ignite the organic matter on a hot plate at not over  $550^{\circ}$ . When the ash is grey or white, extract with 1 cc. of 6 *N* hydrochloric acid. If carbon is left, reash the residue, adding potassium nitrate, and extract again. Wash the residue on the filter. Treat the extract or extracts and washings with excess of bromine-water to oxidize iron and boil off the excess bromine. Dilute to 10–20 cc. and add 10 per cent ammonium acetate solution until the solution is neutral to methyl orange. Filter to remove the turbidity of iron phosphate and wash the filter. If the precipitate is large, dissolve in the minimum amount of 6 *N* hydrochloric acid and dilute to 10–20 cc. Neutralize to methyl orange with a 10 per cent ammonium acetate solution and reprecipitate by addition of excess phosphate.

To the combined filtrates, neutral to methyl orange, add 0.5 mg. of copper as a standard copper sulfate solution.<sup>13e</sup> Pass in hydrogen sulfide until precipitation of copper and zinc sulfides is complete. Filter and transfer the filter to the flask in which the sulfides were precipitated. Add 0.2–0.5 cc. of 6 *N* nitric acid and heat almost to boiling, with agitation, to dissolve the copper and zinc sulfides. Filter the zinc-copper solution and wash the filter.

If the amounts of calcium and magnesium in the original sample were large, add 10 per cent sodium acetate solution to this nitric acid solution until neutral to methyl orange and again precipitate and dissolve the sulfides.

Evaporate the solution of copper and zinc nitrates just to dryness. Dissolve the residue in 6 *N* hydrochloric acid and again evaporate to dryness to drive off nitric acid. Repeat. Dissolve the chlorides of copper and zinc in 0.2 cc. of 6 *N* hydrochloric acid and add 5 cc. of water. Precipitate copper sulfide by hydrogen sulfide, filter and wash. Evaporate the filtrate and washings almost to dryness to leave the sample only slightly acid. Dilute to a known volume and use as sample.

<sup>13e</sup> See p. 150.

**Procedure**—As reagent use a urobilin, which need not be of exactly known concentration, preliminary experiments have indicated the zinc equivalent, about 0.015 mg. per cc.

To the sample solution containing 0.001–0.01 mg. of zinc, and to a series of standards prepared from a solution containing 0.01 mg. per cc.,<sup>13f</sup> add the necessary amount of urobilin solution, usually about 1 cc. This should give a pink rather than a yellow color; excess of the reagent decreases the sensitivity. Dilute the tubes to 50 cc. with purified alcohol and mix. Render each alkaline with a drop of concentrated ammonium hydroxide at the same time. The solutions become yellow with a green fluorescence. Compare them after 10 minutes, but within 30 minutes after rendering alkaline.

---

<sup>13f</sup> See p. 350.

## CHAPTER XXXI

### TITANIUM AND CERIUM

#### TITANIUM BY HYDROGEN PEROXIDE

THE most common method of determining titanium colorimetrically is by oxidation with hydrogen peroxide in acid solution. Such a solution is colored yellow.<sup>1,2</sup> The reaction is often assumed to be due to formation of pertitanic acid,  $\text{H}_4\text{TiO}_5$ , but is more apt to be from an ion like  $[\text{TiO}_2(\text{SO}_4)]^-$ .<sup>3</sup> Vanadium, chromium, tungsten, molybdenum, and traces of fluorides must be removed before the determination. Fusion of the sample with sodium carbonate and a small amount of sodium nitrate followed by leaching with hydrogen peroxide solution eliminates chromium, vanadium and molybdenum.<sup>4</sup> Titanium remains in the residue and is soluble in sulfuric acid. Zirconium, tantalum and columbium do not interfere. Ferric salts which also produce a yellow color in acid solution are removed or allowed for. A correction may be applied, since 0.1 gram of ferric oxide in 100 cc. of 5 per cent sulfuric acid solution is reported<sup>5</sup> to be equal to 0.2 mg. of titanium dioxide when oxidized by hydrogen peroxide.

The presence of phosphoric acid causes a slight fading of the yellow color produced by the reaction. This may be overcome by the addition of 1 cc. of 0.1 per cent uranium acetate solution to each 0.1 mg. of sample.<sup>6</sup> Uranium has also been reported as an interfering element.<sup>7</sup>

At concentrations under 3 mg. per 100 cc., difficulties with colorimeters have caused apparent deviations<sup>8</sup> from Beer's law, which were

<sup>1</sup> A. Weller, *Ber.* 15, 2592-9 (1882).

<sup>2</sup> W. A. Noyes, *Z. anal. Chem.* 5, 39-41 (1891).

<sup>3</sup> Robert Schwartz, *Z. anorg. allgem. Chem.* 210, 303 (1933).

<sup>4</sup> William M. Thornton, "Titanium," p. 133. Chemical Catalogue Co., New York (1927).

<sup>5</sup> W. F. Hillebrand, *U. S. Geol. Survey Bull.* 700, 160 (1919).

<sup>6</sup> F. G. Germuth, *J. Am. Chem. Soc.* 50, 1910 (1928).

<sup>7</sup> William M. Thornton, "Titanium," p. 134. Chemical Catalogue Co., New York (1927).

<sup>8</sup> H. Ginsberg, *Z. anorg. allgem. Chem.* 209, 105-12 (1932).

eliminated for as low as 0.2 mg. per 100 cc. by the measurement of absolute color.<sup>9</sup> The photoelectric colorimeter has been applied.<sup>10</sup>

The general method used with the different types of samples is the same and consists of obtaining the titanium, usually as sulfate, in a solution containing 5 per cent of acid and duplicating the color by addition of a standard solution. In rock and mineral analysis it may be applied to the solution titrated for total iron. Titanium in materials other than

below may be determined in the same way by a suitable treatment of the sample so as to obtain the titanium in solution without interfering substances.

At least 5 per cent of acid is necessary to prevent hydrolysis to metatitanic acid<sup>11</sup> or to a basic sulfate.<sup>12</sup> The presence of alkali sulfates in large amounts causes bleaching<sup>13</sup> unless a large excess of sulfuric acid is present. This has been confirmed by William Blum.<sup>14</sup>

The method has also been recommended<sup>15</sup> for analysis of bauxite by comparison with a series of standards. It is claimed that the standards keep for 8 days. Simultaneous application of the duplication method for titanium and vanadium in cast iron and steel has been reported.<sup>15a</sup> In that case persulfate was used for preliminary oxidation.

The range of maximum sensitivity is 1.5 to 20 mg. of titanium oxide per cc. of solution. The minimum perceptible difference is 6.5 per cent; and averaging, the error by this method

is not more than 1 per cent when titanium dioxide is present per 100 cc. the color is not produced by the dissociation.<sup>17</sup> Therefore the method is applicable to dilute solutions. A colorimeter may be used for solutions containing less than 2 per cent.<sup>18</sup>

*m. Ch*  
*nüller,*  
*J. Am. Chem.*  
*tg. 31, 263 (1907).*  
*J. Sci. 4, 28, 119 (1909).*  
*S. Geol. Surv. Bull. 700, 156*

<sup>10</sup> R. C. Wells, *J.* 33,

<sup>17</sup> H. Ginsberg, *Z. anorg. allgem. Ch.* 62-7 (1931).

<sup>18</sup> G. P. Pamfil, *Mon. Sci.* 73, 641-4



about 5 grams of sodium carbonate.<sup>19, 19a</sup> Extract the fused mass with hot water, then add 1:1 hydrochloric acid gradually until in excess. Evaporate to dryness and moisten with concentrated hydrochloric acid. Add a few drops of water and heat on the water bath for 10 to 30 minutes. Add water and filter. The precipitate is mostly silica but retains some iron and titanium. Volatilize the silica by treating the precipitate in a platinum crucible with a few drops of 1:1 sulfuric acid, then adding a few cc. of hydrofluoric acid. Evaporate and ignite strongly. Repeat the evaporations with sulfuric acid several times.

To the hot filtrate from the precipitate of silica add dilute ammonium hydroxide to faint alkalinity. Filter and wash the precipitate with a 2 per cent ammonium chloride solution. Transfer to the crucible containing the titanium and iron left after the volatilization of silica. Fuse the combined precipitates<sup>19</sup> with about 5 grams of sodium carbonate for 2 or 3 hours, let cool and transfer to a beaker with 200 to 250 cc. of boiling water. Heat on the water bath until disintegration is complete. Filter off the iron and titanium oxides, wash, dry, and fuse again with sodium carbonate, finally collecting the iron and titanium precipitate free of other metals.

Fuse the mixed oxides with 15 to 20 parts of potassium acid sulfate until completely dissolved. Let cool with the crucible covered. Transfer to a beaker with about 75 cc. of boiling 5 per cent sulfuric acid. Heat on the water bath until solution is complete. Transfer to a 100 cc. graduated tube, let cool, add 5 cc. of a concentrated solution of potassium persulfate, and 5 per cent sulfuric acid until a total volume of 95 cc. is obtained. This oxidizes ferrous to ferric ion but does not oxidize titanium. Into a second graduated tube put 85 cc. of 5 per cent sulfuric acid and add a concentrated solution of ferric sulfate until the colors match in the same volume. Dilute the standard to 90 cc.

*Silicate Minerals.*<sup>21</sup> *Alternative Procedure.* Treat 0.5 gram of finely ground sample with 1 cc. of 1:1 sulfuric acid and 10 cc. of hydrofluoric acid. Evaporate to dryness and ignite. Cool and pulverize. Fuse with 4 grams of potassium pyrosulfate for 10 minutes at a temperature at which sulfur trioxide fumes are not given off. Heat further to an orange color, avoiding loss of sulfur trioxide as far as possible. Powder the residue and disperse in 50 cc. of water.

Filter into a Nessler tube and wash with water until a volume of 90

<sup>19</sup> W. F. Hillebrand, *U. S. Geol. Survey Bull.* 700, 97 (1919).

<sup>19a</sup> Cf. N. I. Budakov, *J. Applied Chem. U. S. S. R.* 7, 233-4 (1934).

<sup>21</sup> A. Cavazzi, *Ann. chim. applicata.* 12, 105 (1919).

cc. is obtained. Add 5 cc. of concentrated sulfuric acid. As a standard mix 85 cc. of water and 5 cc. of concentrated sulfuric acid. Dissolve 4 grams of potassium pyrosulfate in this standard.

*Glass.*<sup>21a</sup> Treat with sulfuric and hydrofluoric acids as for silicate minerals.

*Steel. Titanium content more than 0.02 per cent.* Vanadium, chromium, tungsten, molybdenum and nickel must be absent. Transfer 1 gram of sample drillings<sup>22</sup> to a beaker and treat with 15 cc. of 1:3 sulfuric acid.

Heat until dissolved and add 5 cc. of 1:1 nitric acid to oxidize the iron. Boil until all brown fumes disappear. Treat in the same way 1 gram of plain steel free from titanium. This serves as a standard. Cool the two solutions to room temperature and transfer to two similar comparison tubes. Some authors<sup>23,24</sup> recommend the use here of 5 cc. of 30 per cent phosphoric acid to remove the color due to iron. Walton states that the presence of phosphoric acid also reduces the color produced by titanium. Dilute the sample to 45 cc. and the standard to about 40 cc.

*Steel. Titanium content less than 0.02 per cent.* Use 2 grams of sample steel drillings<sup>25</sup> and 2 grams of nontitanium steel containing the same alloying elements as the standard. Add to each 50 cc. of concentrated hydrochloric acid and heat until dissolved. Add 4 cc. of 1:1 nitric acid. Evaporate to 10 cc., cool and pour into separatory funnels. Wash out the containers with 15 cc. each of 2:1 hydrochloric acid. Extract the bulk of the iron with 50 cc. of alcohol-free ether. Dilute the aqueous solutions with 225 cc. of hot water. Add an excess of ammonium hydroxide and boil. Let settle, filter and wash the precipitate with hot water. Dissolve the precipitate with 10 cc. of 1:5 sulfuric acid. Pour into comparison tubes, filtering out any paper present. Dilute the sample to 20 cc. and the standard to about 15 cc.

*Cast Iron.* Follow the methods as given for steel containing less than 0.02 per cent titanium. The following modification is necessary because of an insoluble residue left after the acid treatment.<sup>25</sup> Filter the acid solution. Ignite the residue in a platinum crucible. Add a few drops of 1:1 sulfuric acid and 1 cc. of hydrofluoric acid to volatilize the silica, and ignite. Repeat the evaporation with small portions of sulfuric acid.

---

<sup>21a</sup> Gantois, Lejeune, Noel and Scohy, *Bull. soc. chim.* 43, 545-57 (1934).

<sup>22</sup> The Chemists' Committee of the U. S. Steel Corp., "Methods for the Commercial Sampling and Analysis of Alloy Steels," p. 69 (1915).

<sup>23</sup> T. Dieckmann, *Z. anal. Chem.* 60, 231 (1921).

<sup>24</sup> J. H. Walton, Jr., *J. Am. Chem. Soc.* 29, 481-5 (1907).

<sup>25</sup> C. R. McCabe, *Ind. Eng. Chem.* 5, 735-7 (1913).

Fuse with 1 gram of sodium carbonate. Dissolve the fusion in 10 cc. of 1:5 sulfuric acid. Add this to the main solution and proceed as with steel.

The use of hydrochloric acid in place of sulfuric acid in the determination of iron and steel is recommended by some investigators.<sup>27</sup> They report a more intense color development with hydrochloric acid in solutions containing 1 mg. or more of titanium.

*Pig Iron.*<sup>28</sup> Transfer a 5 gram sample to a covered beaker, add 50 cc. of concentrated hydrochloric acid and digest until decomposition is complete. Most of the titanium remains with the insoluble residue of silica and graphitic carbon. Filter and wash with hot water. Ignite the precipitate and volatilize the silica with 1 cc. of hydrofluoric acid in the presence of a few drops of sulfuric acid. Repeat the evaporations with small portions of sulfuric acid. The amount of titanium in the filtrate is usually negligible but may be recovered by diluting to 250 cc. and precipitating titanous acid with sodium thiosulfate as in the gravimetric determination of titanium in the standard solution.<sup>29</sup> Ignite the precipitate in the crucible containing the residue from the volatilization of silica.

Fuse the total residue with 4 grams of sodium carbonate. Disintegrate the fused mass in boiling water. Collect the insoluble sodium titanate on a filter and wash with hot water containing a little sodium carbonate. Dissolve any residue adhering to the crucible with 5 cc. of hot 1:1 sulfuric acid. Transfer this and the filter paper with its precipitate to a beaker, washing out the crucible with hot water. Heat the beaker until the titanium dissolves. Remove the paper, rinsing it with hot water. Filter out any shreds of paper.

Dilute the sample to nearly 45 cc. with distilled water. If too much titanium is present dilute to a definite volume with 5 per cent sulfuric acid and aliquot. Transfer the solution to a color comparison tube. In a similar tube take 40 cc. of 5 per cent sulfuric acid as a blank.

*Ferrosilicon.* Dissolve 5 grams of the alloy in a 1:1 mixture of nitric and hydrofluoric acid. Add 10 cc. of sulfuric acid and evaporate to sulfur trioxide fumes.<sup>30</sup> Cool, moisten with water and add hydrofluoric acid to volatilize the silica. Ignite strongly. Dissolve in 50 cc. of 1:1

<sup>27</sup> O. L. Barnebey and R. M. Isham, *J. Am. Chem. Soc.* 32, 957-62 (1910).

<sup>28</sup> The Chemists' Committee of the U. S. Steel Corp., "Methods for the Commercial Sampling and Analysis of Pig Iron," p. 30 (1912).

<sup>29</sup> See pp. 361-2.

<sup>30</sup> A. A. Blair, "The Chemical Analysis of Iron," 8th ed., p. 238. J. B. Lippincott Co., Philadelphia, Pa. (1918).

hydrochloric acid and dilute to 300 cc. with hot water. Add a few drops of bromine-water and a slight excess of 1:1 ammonium hydroxide. Boil 1 to 2 minutes, filter and wash the precipitate with hot water. Dissolve in hot 1:3 hydrochloric acid. Reprecipitate with 1:1 ammonium hydroxide, boil and filter through the same filter paper. The precipitate contains aluminum, iron, titanium and manganese.

Dissolve the precipitate in hot 1:3 hydrochloric acid and evaporate the solution to a syrup. Extract the iron with ether as given under Rutile and Iron Ores.<sup>31</sup> Aluminum, titanium and chromium are in the acid solution. Evaporate ether from the acid solution and add 1:1 ammonium hydroxide until a faint permanent precipitate is formed. Redissolve this in 1:1 hydrochloric acid, using 2 or 3 drops excess of acid. Heat to boiling and add a few drops of 10 per cent ammonium bisulfite solution, and a few drops of 1 per cent phenylhydrazine solution. Filter and wash with cold water. Ignite the precipitate and fuse with 1 gram of potassium bisulfate. Cool and dissolve in 50 cc. of 5 per cent sulfuric acid. Dilute to 95 cc. with 5 per cent sulfuric acid. The standard consists of 90 cc. of 5 per cent sulfuric acid.

*Rutile and Iron Ores.* Moisten a 1 gram sample in a platinum crucible with water. Add a few drops of concentrated sulfuric acid, and 1 cc. of hydrofluoric acid.<sup>27</sup> Heat until sulfur trioxide fumes are no longer given off. Repeat the evaporation with sulfuric acid several times. Add 5 to 10 grams of sodium carbonate and 0.2 gram of sodium nitrate. Fuse for at least 30 minutes. Cool and place the crucible and cover in a beaker with hot water. Heat until disintegrated. Iron oxide and sodium titanate remain as insoluble material. Remove the crucible and dissolve any residue with hot 1:1 hydrochloric acid. Filter the contents of the beaker and wash with hot water. Dissolve the precipitate in 1:1 hydrochloric acid and add the acid washings from the crucible. Heat until solution is complete and a volume of 20 cc. remains. Cool, add 2 cc. of concentrated hydrochloric acid and transfer to a separatory funnel. Rinse the beaker with 10 cc. of 1:1 hydrochloric acid and add. Add an equal volume of peroxide- and alcohol-free ether saturated with concentrated hydrochloric acid. Shake and let stand for 10 minutes. Draw off the aqueous layer and repeat the ether extraction 2 or 3 times, or until the ether layer is no longer green. Rinse the ether layers with 10 cc. of 1:1 hydrochloric acid and add this to the aqueous portion. Heat the acid solution to expel the ether and cool. Transfer to a comparison tube and

<sup>31</sup> See below.

dilute to 95 cc. with distilled water. Into another comparison tube introduce 40 cc. of 1:1 hydrochloric acid and 50 cc. of water.

Walton recommends fusion of iron ore, limestone, or fireclay with sodium peroxide as a flux.<sup>24</sup> This makes unnecessary the later addition of hydrogen peroxide. Titanium forms a soluble compound, and can be separated from the iron by filtration. The method is not as accurate as that given above as a part of the titanium remains behind with the iron precipitate. It is given as a separate method.

**Pigments.**<sup>34</sup> Treat 1 gram of pigment with 25 cc. of concentrated sulfuric acid and 5 grams of sodium sulfate. Heat until decomposition is complete. Continue this heating until sufficient sulfur trioxide has been volatilized so that only about 10 grams remains. Cool and dilute with water to a volume of 200 cc. Filter and use a 50 cc. aliquot of the filtrate diluted to 90 cc. as sample. As standard for duplication mix 1.25 grams of sodium sulfate and 0.75 cc. of concentrated sulfuric acid with water and dilute to 90 cc.

**Reagent**—Usually commercial 3 per cent hydrogen peroxide is used. The peroxide solution may also be prepared from sodium peroxide according to the following procedure.

Add 7 grams of sodium peroxide in small portions to 25 cc. of 1:1 sulfuric acid. After each addition let cool to room temperature. Dilute with water to 100 cc. This gives a 3 per cent solution of hydrogen peroxide.

**Procedure**—Add 5 cc. of 3 per cent hydrogen peroxide to both sample and standard. To the standard then add sufficient standard titanium solution to match the color of the sample. Adjust the volume of standard to equal that of the sample. If the color of the sample is too faint add a measured volume of standard solution and again compare.

**Standard Titanium Solution**—*From Titanic Acid.* Fuse 1.05 grams of ignited pure titanite acid with 10 grams of sodium carbonate. Digest with 100 cc. of hot water until the soluble alkali is completely dissolved. Filter and wash the insoluble sodium titanate with hot water containing a little sodium carbonate. Dissolve the titanate from the filter with 100 cc. of 1:1 sulfuric acid and dilute to 1 liter with water. One cc. of this solution contains about 0.6 gram of titanium. Remove an aliquot and determine the titanium gravimetrically as below.

<sup>34</sup> B. V. Health, *Proc. Soc. Chem. Ind. Victoria* 31, 531-3 (1931).

Add concentrated ammonium hydroxide until a precipitate appears which redissolves on stirring. Add 2 per cent ammonium hydroxide until a faint permanent precipitate is obtained. Dissolve this by adding 15 cc. of 1:3 hydrochloric acid. Add 100 cc. or more of 20 per cent sodium thiosulfate solution and stir until the iron is completely reduced and free sulfur begins to separate. Boil for 10 minutes and filter. Wash with 2 per cent acetic acid, ignite and weigh the titanium dioxide. From this weight calculate the amount of titanium sulfate in the standard solution.

*From Titanium Dioxide.*<sup>36</sup> Prepare ammonium bisulfate by mixing chemically equivalent quantities of ammonium sulfate and sulfuric acid. Fuse 1 gram of pure titanic oxide with about 10 g. of the above. Take up with 200 cc. of 1:4 sulfuric acid and dilute to 1 liter with distilled water. Standardize by evaporation of a measured portion to dryness in platinum and ignite to constant weight. If necessary add ammonium carbonate to assist in volatilizing sulfuric acid.

*From Ferrotitanium.*<sup>35</sup> Treat 100 grams of ferrotitanium with 50 cc. of concentrated hydrochloric acid. Heat and add 5 cc. of 1:1 nitric acid. Evaporate to half volume, add 20 cc. of water and filter. Repeat the treatment of the filtrate until sufficient ferrotitanium is dissolved, usually about 10 times.

Evaporate until titanic acid begins to separate. Extract in a separatory funnel with ether until free of iron, as shown by testing with potassium thiocyanate. To the acid solution add 150 cc. of 1:1 sulfuric acid, heat and filter. Dilute to 700 cc., add ammonium hydroxide in excess and boil. Let settle and decant. Wash the precipitated titanic acid free of chlorides by decantation. Dissolve the titanic acid in 100 cc. of 1:1 sulfuric acid and dilute to 1 liter. Determine titanium in a 100 cc. aliquot according to the method outlined for the standard from titanic acid.

*Artificial.* Methyl orange at a concentration of 0.01 per cent has been recommended as a stock solution for preparation of permanent standards.<sup>35</sup> This is subject to the usual objections, and in addition titanium colors may vary due to variation in iron content, if ether extraction of iron is not complete.

#### TITANIUM BY SODIUM PEROXIDE FUSION

Instead of producing the titanium sample solution and adding hydro-

<sup>36</sup> F. P. Dunnington, cf. William M. Thornton, "Titanium," p. 128. Chemical Catalogue Co., New York (1927).

<sup>35</sup> A. Gautier, *Chimiste* 2, 2 (1911); *Rev. gen. chim.* 14, 16 (1911); *Ann. chim. anal. chim. appl.* 12, 135-7 (1930).

gen peroxide, the fusion may be carried out with sodium peroxide.<sup>24</sup> In that case the color develops as soon as acid is added. The method is particularly applicable to minerals and ores. The precautions are the same as for the previous method and the accuracy would be the same, provided titanium is all in solution.

**Procedure**—For a mineral containing 1 to 5 per cent of titanium dioxide weigh about 1 gram of sample. This can be varied with the titanium content. Mix with 8 grams of sodium peroxide in an iron crucible and fuse. When fusion is complete, cool and dissolve in 200 cc. of water. Without filtering add 15 cc. of 1:1 sulfuric acid and 6 cc. of 50 per cent phosphoric acid. Dilute to 250 cc. and transfer 100 cc. to a comparison cylinder.

In a similar cylinder place 6 cc. of 1:1 sulfuric acid and 2.4 cc. of 50 per cent phosphoric acid. Dilute to 90 cc. with water. Add about 3 grams of sodium peroxide, mix well and add standard titanium solution until the color of the sample is duplicated. If necessary to get a color match a small amount of ferric salt solution may be added to the standard. Finally equalize the volumes of the two solutions.

The color fades more rapidly than when it is developed with hydrogen peroxide, presumably due to the salts present.

#### TITANIUM BY THYMOL

This method permits the determination of from 0.2 to 2.5 mg. of titanium dioxide. The color produced by titanium with thymol is about 25 times as intense as that produced by the hydrogen peroxide method. The reaction with thymol gives a red or reddish yellow color, according to the concentration.<sup>39</sup> The only substance apt to be present which would alter the color is tungstic acid. The method has been found accurate to 5 per cent.

**Reagent**—Dissolve 5 grams of thymol in 5 cc. of glacial acetic acid. Dilute with concentrated sulfuric acid to 100 cc. Protect this solution from sunlight. The proportion of thymol used to titanium dioxide should be at least 60 to 1 by weight. If the thymol is dissolved directly in the sulfuric acid an interfering yellow color is produced.

<sup>39</sup> V. Lehner and W. G. Crawford, *Orig. Com. 8th Intern. Cong. App. Chem.* 1, 285 (1912); *J. Am. Chem. Soc.* 35, 141 (1913).

**Procedure**—Fuse a 0.5 gram sample of titanium-bearing substance containing 1–10 mg. of titanium dioxide with 5 grams of potassium acid sulfate. Dissolve the fusion in concentrated sulfuric acid and treat with 20 cc. of 5 per cent thymol in concentrated sulfuric acid. Make up to 100 cc. with concentrated sulfuric acid. If necessary take a 10 cc. aliquot, add 20 cc. of reagent and dilute to 100 cc. with concentrated sulfuric acid. Compare with a standard titanium solution similarly treated at room temperature. Sample and standard must be at the same temperature. Increase of temperature decreases the color. At 100° it is destroyed. The dilution method is suitable for comparison, using concentrated sulfuric acid the acid content gets below 70 per cent the color becomes that proportional to the t

**Standard**—Fuse 0.1 gram of titanium dioxide with 2 grams of potassium acid sulfate. Dissolve in concentrated sulfuric acid and dilute to 100 cc. of titanium dioxide per cc. of the same. This solution contains 0.001 per cent of titanium dioxide per cc.

#### TITANIUM BY SALICYLIC ACID

Alkaline titanates react with salicylate.<sup>40</sup> Interference from the presence of very small amounts of columbium and thorium. Tan because of the interference this method is of little practical determination of nearly all earths. form an intensely yellow color developed occurs in the presence of niobium, molybdenum, zirconium, and cerium. The color does not affect the color. Because of the interference of these contaminants of titanate oxide this method may be used for an accurate determination of titanium or for its detection in acid earths.

Other investigators<sup>39</sup> have found that sulfuric acid detects titanium to the titanium content.

**Procedure**—Fuse a 0.5 gram sample with 5 grams of potassium carbonate. Take up the melt with warm water and dilute to 100 cc. Fuse any residue again in order to have a complete fusion and add to the main volume. Dilute to 500 cc. If the color is orange rather than yellow remove 100 cc. and dilute to 1 liter. Compare with a series of standards. As little as 0.05 mg. per 100 cc. gives a faint yellow or distinguishable from a blank. A suitable series of standards containing 0.1, 0.5, 1, 0.5, 0.1, and 0.05 mg. per 100 cc. If the color of the more



dilute solutions fades it can be brought back by addition of more salicylic acid.

**Standard**—Treat 0.5 gram of pure titanium dioxide in the same way as the sample, and dilute 100 cc. of the solution to 1 liter. These contain 1.0 and 0.1 mg. of titanium dioxide per cc. From these solutions prepare standards.

#### TITANIUM BY DIHYDROXYMALEIC ACID

When hydrogen peroxide is added to a solution containing titanium and vanadium a red color is produced by both. If dihydroxymaleic acid is added, only the titanium gives such a color.<sup>42</sup> Prepare suitable curves of the results of such treatment using a tintometer for reading the colors.

By comparing the effect produced by hydrogen peroxide on a solution containing both titanium and vanadium and the effect produced by dihydroxymaleic acid on another portion of the same solution the concentrations may be read from the chart made for a particular tintometer and thickness of cell.

#### TITANIUM BY AMMONIUM MOLYBDATE

Sodium titanate gives a pale greenish yellow color with ammonium molybdate and nitric acid.<sup>43</sup> The absence of phosphorus and silica is essential. Vanadium does not interfere. The color takes twelve minutes to develop and does not fade in an hour.

**Procedure**—Add 4 cc. of 5 per cent neutral ammonium molybdate solution and 2 cc. of 1:5 nitric acid to 50 cc. of neutral titanium solution. Dilute to 100 cc. and, after the color is fully developed, compare with a series of standards similarly prepared.

#### TITANIUM BY GALLIC ACID

Substantial amounts of titanium give a red-brown color with gallic acid.<sup>43a</sup> Lesser amounts give a yellow or greenish yellow. The color is deepened by sodium acetate and is suitable for colorimetric estimation.

<sup>42</sup> J. W. Mellor, *Trans. Ceram. Soc. England* 12, 33 (1913).

<sup>43</sup> A. G. Woodman and L. L. Cayvan, *J. Am. Chem. Soc.* 23, 105 (1901).

Nath Das-Gupta, *J. Indian Chem. Soc.* 6, 855-63 (1929).

The results are reported <sup>43b</sup> to be as accurate as those by the hydrogen peroxide method. It will detect 1 p.p.m.

**Procedure**—To 1 cc. of the sample solution add 0.5 cc. of 1 gallic acid solution and 0.5 cc. of 5 per cent ammonium Mix and compare with a series of standards.

#### CERIUM BY GALLIC ACID

modified

blue-violet. Thorium, lanthanum a . Iron, chrom-  
ium, aluminum, manganese, nickel, cobalt and do not  
fere. Atmospheric oxidation must be prevented.

**Procedure**—Mix 2.7 cc. of sample aining 0.03–0.07 mg.  
of cerium with 2.7 cc. of 0.02 per cent lution. Add 2 cc. of  
surface.

oxide per 100 cc. Mix carefully :  
and 10 cc., with the protective layer above the  
ies of standards similarly developed from a  
known cerium solution.

**Standard**—Dissolve  
and dilute to 1 liter. F

cc. of this to 50 cc.,  
cc. specified  
above.

b. 3,

<sup>43c</sup> F. M. Shemyakin, *Z. anorg. allgem. Chem.* 211, 212 (1933).

## CHAPTER XXXII

### VANADIUM

#### VANADIUM IN THE ABSENCE OF TITANIUM BY HYDROGEN PEROXIDE

A SOLUTION of vanadium is colored reddish brown by hydrogen peroxide. The color is influenced by the presence of iron, strong acids, and excess hydrogen peroxide. Tungsten, chromium, nickel, molybdenum, etc., also interfere. Interference, except by titanium, may be overcome by using as a standard a steel free from vanadium and containing the same other constituents as the sample. Another method is to determine the chromium and nickel which give a green color in the unknown and then add corresponding amounts to the standard. Iron is sometimes removed by extraction with ether.<sup>1</sup> The vanadium must be in the highest form of oxidation. Simultaneous estimation of titanium and vanadium in iron and steel by duplication has been reported.<sup>1a</sup>

By addition of phosphoric acid an insoluble compound is formed with tungsten.<sup>2</sup> Interference from combined carbon is eliminated by treatment with persulfate or permanganate. The results are accurate to about 0.04 per cent of vanadium.<sup>3</sup>

**Sample—Steel.**<sup>4</sup> Treat 1 gram of steel with 40 cc. of 1:4 sulfuric acid and heat. If tungstic acid is precipitated, filter and wash. To the filtrate add 20 cc. of 1:1 sulfuric acid, and evaporate to sulfur trioxide fumes. Dissolve the residue in hot water. To this, or to the solution obtained directly by heating with sulfuric acid, add 5 cc. of 1:1 nitric acid. Boil until the nitric oxide vapors have disappeared. Add 10 cc. of a 5 per cent solution of ammonium persulfate to oxidize organic matter. Boil about 3 minutes to decompose excess persulfate, and let cool.

<sup>1</sup> C. R. McCabe, *Chem. News* 104, 194 (1911); *J. Ind. Eng. Chem.* 5, 735-7 (1913).

<sup>1a</sup> P. Duez, *Bull. assoc. tech. fonderie* 8, 570-1 (1934).

<sup>2</sup> A. Kropf, *Z. angew. Chem.* 35, 366 (1922).

<sup>3</sup> J. A. Pickard, *Chem. World* 2, 341-2 (1913).

<sup>4</sup> The Chemists' Committee of the U. S. Steel Corporation, "Methods for the Commercial Sampling and Analysis of Alloy Steels," p. 69. U. S. Steel Corporation (1915).

As standard treat 1 gram of steel containing no vanadium, or a carefully determined amount of vanadium, exactly like the unknown. If no vanadium is present add a standard solution to approximate the concentration of the sample.

*Steel, Alternative Method.* Weigh 0.2 gram if the vanadium is less than 0.5 per cent, or 0.1 gram if over that amount. Prepare a mixture of 1 part syrupy phosphoric acid, 6 parts concentrated sulfuric acid and 33 parts of water. Add 3 cc. of this mixture. Samples containing chromium dissolve readily to a clear solution. Tungsten remains as a black residue. Add 1 cc. of 1:1 nitric acid and heat until no more nitric oxide fumes are given off. Cool and add 1 cc. of 10 per cent ammonium persulfate solution. Heat until evolution of gas ceases. Cool and let stand for phosphomolybdate to precipitate, if necessary.

As standard dissolve a steel of known composition having similar interfering elements. If such a steel is not available dissolve a plain steel and add standard solutions of chromium and nickel to equal the amounts previously found in the sample.

*Steel. Chromium Absent.*<sup>1</sup> Place 2 gram portions of sample and standard steel free from vanadium in flasks. Add 40 cc. of 1:1 nitric acid to each and heat until solution is complete. Add about 0.1 gram of potassium permanganate to each to oxidize carbon and digest about 5 minutes. Prepare a solution of ammonium bisulfite by mixing equal volumes of a saturated solution of sulfur dioxide and 1:1 ammonium hydroxide. Add this until the solution is clarified and boil until sulfur dioxide is completely expelled. Incomplete removal will give low results. Transfer to Nessler tubes and add an amount of standard vanadium to the standard steel, such as is indicated by experience with the type of steel. Dilute to 45 cc. each. More standard solution may be added later if necessary.

*Steel. Chromium Present.*<sup>1</sup> Place 2 gram samples of chromium-free standard and sample steel in beakers. The chromium in the sample must be known. Add sufficient 0.1 per cent potassium bichromate solution to the standard to equal the chromium content of the sample. To each add 80 cc. of 1:3 sulfuric acid and heat. When solution is nearly complete add 25 cc. of concentrated nitric acid to each and continue to boil for 10 minutes. This is necessary to oxidize vanadium completely.

Cool, transfer to Nessler tubes and add standard solution to the standard steel. Add water to dilute to 45 cc. each. Particular attention must be given to the color when developed to see that the colors are of the same character.

*Steel, Tungsten, Chromium, Titanium and Molybdenum Present.*<sup>7,8</sup> Weigh a 2 gram sample and transfer to a beaker. Dissolve in 40 cc. of 1:1 nitric acid and filter from precipitated tungstic acid. Render the filtrate just alkaline to litmus with 1:1 ammonium hydroxide and evaporate to dryness. Take up with 20 cc. of 1:1 nitric acid and filter out the tungstic acid. Evaporate to about 10 cc. Pour into a separatory funnel and rinse the dish with 10 cc. of 1:1 hydrochloric acid in several portions. Add 50 cc. of alcohol-free ether and extract the bulk of the iron. Draw off the acid solution and a little ether with it. Titanium, vanadium and chromium are in the acid layer. Molybdenum is in the ether layer.

Evaporate the acid solution nearly to dryness. Add 5 cc. of concentrated nitric acid and again evaporate nearly to dryness. This removes all chlorides. Add 20 cc. of concentrated nitric acid and heat to boiling. Add 2 grams of potassium chlorate and continue boiling for about 1 minute. Chromium and manganese are oxidized. Dilute to about 250 cc. Manganese goes into solution. Heat to boiling and slowly neutralize by addition of 1:1 ammonium hydroxide. Iron is precipitated and occludes the vanadium. Titanium is also precipitated but chromium is in solution. Filter and wash with hot water.

Transfer the filter to a dish and dissolve in 9 cc. of 1:5 hydrochloric acid by heating. Do not add more acid. Filter from paper fibers and manganese and wash. Transfer to a comparison tube and dilute to about 40 cc. The solution is colored with ferric chloride. Add 1.0 cc. of hydrofluoric acid and mix to discharge the ferric chloride color. This is not effective if much excess hydrochloric acid is present. Dilute to 45 cc. Titanium is also present in this solution but hydrofluoric acid prevents it from interfering.

As standard for use with this sample mix 9 cc. of 1:5 hydrochloric acid, 0.1 cc. of 5 per cent ferric chloride solution and 1.0 cc. of hydrofluoric acid. Add an estimated requirement of standard vanadium solution and dilute to 45 cc.

**Reagent**—The commercial 3 per cent solution is quite commonly used and is satisfactory. As an alternative, dissolve 7 grams of sodium peroxide in 25 cc. of 1:1 sulfuric acid and dilute with water to 100 cc.

**Procedure**—Dilute sample and standard to the same volume, 45 cc.

<sup>7</sup> C. R. McCabe, *Chem. Eng.* 13, 243 (1911).

<sup>8</sup> John H. Yoe, "Photometric Methods of Analysis," vol. I, "Colorimetry," p. 391. John Wiley and Co., New York (1928).

or 95 cc. Add 5 cc. of 3 per cent hydrogen peroxide and mix well. Compare by dilution. The extent of this should not exceed 10 per cent because of the difference in color introduced by the varying iron content.

The color of the sample may also be matched by the duplication method, in which case all additions of standard vanadium solution specified are to be omitted.

**Standard Vanadium Solution—From Ammonium Vanadate.**<sup>9</sup> Dissolve 1 gram of ammonium vanadate in 50 cc. of water. Add 20 cc. of 1:1 sulfuric acid, dilute to 1 liter and mix. To standardize, take 2 portions of 200 cc. Add to each 25 cc. of 1:1 sulfuric acid and heat to boiling. Add 30 cc. of aqueous sulfurous acid. Boil off excess sulfurous acid and titrate hot with a standard potassium permanganate solution. Add 50 cc. of dilute nitric acid to the remaining solution and dilute until 1 cc. corresponds to 0.1 mg. of vanadium. The cc. of permanganate times the normality times 0.051 gives the amount of vanadium present.

**From Vanadium Pentoxide.**<sup>2</sup> Dissolve 1.8 grams of commercial vanadium pentoxide in 10 cc. of 10 per cent potassium hydroxide solution. Cool and dilute to about 50 cc. Add 50 cc. of 1:3 sulfuric acid and dilute to 1 liter. Determine the vanadium content of a 200 cc. aliquot by the preceding method and dilute the balance of the solution so that 1 cc. contains 0.05 mg. of vanadium.

#### VANADIUM IN THE PRESENCE OF TITANIUM BY HYDROGEN PEROXIDE

Under controlled conditions the presence of fluorides destroys the yellow color produced by titanium with hydrogen peroxide, and permits the estimation of vanadium by means of the red brown color of the latter in the presence of hydrogen peroxide.<sup>11</sup>

**Sample—Minerals.** Treat a finely ground 1 gram sample with 2 cc. of hydrofluoric acid and a few drops of sulfuric acid. Evaporate substantially to dryness. Add 5 grams of sodium acid sulfate and fuse. Take up the fused mass with 25 cc. of 1:10 sulfuric acid. Filter if necessary.

As standard treat in parallel 1 gram of China clay known to be free from vanadium. Dilute sample and standard to 45 cc. To both sample and standard add 1 cc. of 80 per cent phosphoric acid.

<sup>9</sup> P. Slawik, *Chem.-Ztg.* 34, 648 (1910).

<sup>11</sup> G. Fenner, *Chem.-Ztg.* 42, 403 (1918).

**Procedure**—To sample and standard add 3 per cent hydrogen peroxide in 1 cc. increments until a maximum color is developed in the sample. Add hydrofluoric acid to both in the same way until the color of the sample is reduced to a value which is not reduced by further additions. Dilute the sample to 100 cc. and standard to 95 cc. To the standard add a stock vanadium solution <sup>11a</sup> until the color of the sample is duplicated.

VANADIUM IN THE PRESENCE OF TITANIUM, MOLYBDENUM AND TUNGSTEN,  
BY STRYCHNINE

Vanadium gives a violet color followed by an intense orange with strychnine in concentrated sulfuric acid.<sup>12</sup> Titanium, molybdenum and tungsten do not interfere. Iron prevents the formation of the colored compound and must be removed.

**Sample**—*Steel*.<sup>13</sup> Dissolve 1 gram of steel in 45 cc. of 1:2 nitric acid and add 1 cc. of 10 per cent disodium phosphate solution. Boil until the nitric oxide fumes have disappeared, cool and add carefully 14 cc. of 1:1 ammonium hydroxide. Boil until all ferric hydroxide is redissolved, remove and add 3 cc. of 5 per cent neutral ammonium molybdate solution. Shake. Vanadium is precipitated with the phosphomolybdate. Filter on a small filter and wash free from iron with 2 per cent nitric acid, finally washing with water. Transfer the precipitate to a beaker, add a crystal of potassium chlorate and 20 cc. of 1:1 sulfuric acid. Evaporate to the appearance of sulfur trioxide fumes. To a suitable amount of a standard solution of vanadium <sup>14</sup> add a crystal of potassium chlorate, 20 cc. of 1:1 sulfuric acid, evaporate to the appearance of sulfur trioxide fumes and let cool.

**Minerals**.<sup>12</sup> Fuse a 1 gram sample with 10 grams of sodium carbonate. Extract with water and fuse the insoluble residue again with sodium carbonate. Extract, acidify the two extractions with nitric acid, and make to a definite volume. Treat an aliquot in the same way as the steel sample, starting with the addition of potassium chlorate and sulfuric acid. As standard take an equal aliquot of a solution of 10 grams of sodium carbonate acidified with nitric acid, add the required amount of vanadium solution <sup>14</sup> and dilute to the same volume. Treat in the same way as the sample.

<sup>11a</sup> See p. 370.

<sup>12</sup> A. W. Gregory, *Chem. News* 100, 221 (1909).

<sup>13</sup> J. A. Pickard, *Chem. World* 2, 342 (1913).

<sup>14</sup> See p. 370.

**Procedure**—To each, sample and standard, add 20 cc. of a 0.4 per cent solution of pure strychnine in concentrated sulfuric acid. After 10 minutes compare the two by diluting the darker with concentrated sulfuric acid. The color is permanent for several hours.

#### VANADIUM BY DIPHENYLAMINE

An aqueous solution of diphenylamine gives a violet color with vanadium, much more sensitive than the peroxide reaction.<sup>15</sup> It will detect 0.0025 mg. of ammonium vanadate per cc. The maximum color is obtained at 50°. Small amounts of iron and of nitrates do not interfere. Titanium does not interfere. The reaction is a general one for active oxidizing agents, which must therefore be absent.

**Sample**—*Iron absent.* Render 50 cc. of sample solution just alkaline with 30 per cent sodium hydroxide solution. Add 1 cc. of saturated alcoholic oxalic acid solution and mix well.

*Iron present.* Render a 50 cc. sample just alkaline with 30 per cent sodium hydroxide solution. Add concentrated hydrochloric acid until a definite yellow color is obtained. Then add concentrated phosphoric acid until the yellow disappears.

**Reagent**—Heat 0.2 g. of diphenylamine with 100 cc. of water on a water bath, cool and filter. It is not affected by exposure to air or light.

**Procedure**—Add 5 cc. of concentrated hydrochloric acid and 5 cc. of reagent. A violet color will appear within 2 minutes and is stable for several minutes. Compare after 10 minutes by dilution, with a standard <sup>15a</sup> similarly treated at the same time as the sample.

**Confirmatory Test**—If any question arises as to the color developed being due to vanadium, extract 5 cc. of colored solution with 1 cc. of chloroform or benzene. A yellow color should be extracted.

#### VANADIUM BY AMMONIUM MOLYBDATE

Ammonium vanadate in neutral solution with a neutral solution of

---

<sup>15</sup> Victor L. Meaurio, *Anales soc. quim. Argentina* 5, 185-9 (1917); *Ann. chim. anal.* 23, 47-50 (1918).

<sup>15a</sup> See p. 370.



ammonium molybdate gives a yellow color permanent for several hours.<sup>16</sup> In acid solution the color fades completely in five minutes.

**Procedure**—Add 10 cc. of freshly prepared 10 per cent ammonium molybdate solution to the sample solution in a Nessler tube and dilute to 100 cc. Compare with a standard of similar composition similarly treated. By this means it is possible to detect 0.002 mg. of vanadium pentoxide in 100 cc. of solution.

#### VANADIUM BY PHOSPHOTUNGSTIC ACID

The reaction of vanadium with phosphotungstic acid may be used for estimation of 0.00001 to 0.001 per cent of vanadium.<sup>17</sup> The method is very similar to that with ammonium molybdate.

**Sample—Plant Ash.** Dissolve the residue from dry ashing in 10 cc. of concentrated nitric acid and dilute to about 50 cc. Filter and wash the filter with hot water until free from acid. Precipitate chlorides from the sample solution with 1 cc. of 10 per cent silver nitrate solution and filter. Oxidize the filtrate with 0.8 per cent potassium permanganate solution until destruction of organic matter is complete. Remove manganese dioxide and excess of potassium permanganate by addition of 30 per cent hydrogen peroxide. Boil to destroy the excess of peroxide.

**Magnetite.**<sup>18</sup> Heat 1 gram of finely ground titaniferous magnetite with 15 cc. of concentrated nitric acid and 45 cc. of concentrated hydrochloric acid. Evaporate to dryness and dissolve the residue in 25 cc. of concentrated nitric acid. Evaporate this to about 5 cc., dilute to 25 cc. with hot water and filter. Evaporate the filtrate to dryness and dissolve in 20 cc. of 1:1 nitric acid. Add 2 cc. of 10 per cent silver nitrate solution and boil for several minutes to remove chlorides. Cool and filter.

Add 10 cc. of an 0.8 per cent solution of potassium permanganate to the filtrate and boil to remove any remaining organic matter. Add 30 per cent hydrogen peroxide to remove excess of potassium permanganate and precipitated manganese dioxide. Destroy the excess of hydrogen peroxide by heating to boiling. Cool and dilute to 100 cc. To a 10 cc. aliquot add 18 cc. of 1:1 nitric acid and 10 cc. of 85 per cent phosphoric acid. Dilute to 80 cc.

<sup>16</sup> A. G. Woodman and L. L. Cayvan, *J. Am. Chem. Soc.* 23, 105 (1901).

<sup>17</sup> A. P. Vinogradov, *Compt. rend. acad. sci. (U. S. S. R.)* 1931A, 249-52.

<sup>18</sup> K. Bolshakov, *Tsvetnue Metal.* 6, 487-93 (1931); *Chimie & Industrie* 27, 81 (1932).

As standard for development with this, mix 20 cc. of 1:1 nitric acid, 10 cc. of 85 per cent phosphoric acid and a volume of standard containing 0.2 mg. of vanadium pentoxide.<sup>19</sup> Dilute to 80 cc ment.

**Procedure**—Add 10 cc. of 85 per cent phosphoric acid to the sample and dilute to 80 cc. Add 10 cc. of 10 per cent phosphotungstic acid and dilute to 100 cc. Compare with a standard similarly treated.

---

<sup>19</sup> See p. 370.

## CHAPTER XXXIII

### TUNGSTEN

#### TUNGSTEN AS THE COLLOIDAL OXIDE BY TITANIUM TRICHLORIDE

THIS method may be used for determining tungsten in stanniferous wolframite.<sup>1</sup> Tungstic acid, when reduced with titanium trichloride, gives a blue oxide<sup>2</sup> which will remain in colloidal suspension about one-half hour. More than 0.1 *N* hydrochloric acid lessens the color and it fades completely at 0.5 *N*. Vanadium gives a tungstovanadate very difficult to reduce. Phosphorus gives a precipitate of titanium phosphate. Molybdenum modifies the color and renders it unstable. These elements must therefore be absent. The method is accurate to within 2 per cent. The amount of tungsten in the final solution must not be over 1 mg. per cc., otherwise the oxide will flocculate.

**Sample—Minerals.** Pulverize the mineral carefully and fuse the sample with anhydrous sodium sulfite in porcelain. Treat the fused mass with *aqua regia*. The major part of the tungsten remains as a precipitate of tungstic acid mixed with silica. The rest of the tungsten has been changed to metatungstic acid, which goes into colloidal solution. To recover this, filter and neutralize the filtrate with 1:1 ammonium hydroxide, taking care to avoid excess. If iron is absent in the sample add a few cc. of 1 per cent ferric chloride before neutralizing. This precipitates iron as ferric hydroxide. The iron carries down with it the metatungstic acid. Filter and wash the precipitate well to remove sodium salts. Dissolve on the filter with warm 1:1 hydrochloric acid and evaporate to dryness. Treat the residue with 5 cc. of 1:1 hydrochloric acid. Filter on the same paper. The insoluble tungstic acid retains only a trace of iron oxide. Ash and add to the tungstic acid previously obtained. Test the acid filtrate from which tungstic acid has been separated. It should not give a blue color with titanium trichloride. If color is obtained estimate the trace by comparison with a standard.

<sup>1</sup> A. Travers, *Compt. rend.* 165, 408 (1917).

<sup>2</sup> A. Travers, *Compt. rend.* 166, 416 (1918).

Volatilize the silica with hydrofluoric acid.<sup>2a</sup> Take up the tungstic acid with 2 per cent sodium hydroxide solution and neutralize carefully with *N* hydrochloric acid. Add an excess of about 5 per cent by volume of *N* hydrochloric acid.

*Films.*<sup>3</sup> For films of tungsten, such as deposits on the walls of electric light bulbs, dissolve in 5 cc. of 3 per cent hydrogen peroxide, rendered slightly alkaline with ammonium hydroxide or slightly acid with nitric acid. Very small amounts of tungsten may also be dissolved in 1:3 nitric acid. Transfer the solution to a platinum dish. Add 1 cc. of 1:1 hydrochloric acid and evaporate nearly to dryness. Dilute to a convenient volume with distilled water.

*Filaments.*<sup>3</sup> Add 3 cc. of strong hydrofluoric acid and 0.5 cc. of concentrated nitric acid to the sample in a platinum dish. Heat carefully to avoid loss as the reaction to form tungsten oxytetrafluoride is vigorous. After solution is complete add 1 cc. of 1:1 hydrochloric acid and evaporate nearly to dryness to remove excess hydrofluoric acid. Any hydrofluoric acid remaining will make reduction of the tungstic oxide very difficult. Take up and dilute to a convenient volume with distilled water.

**Procedure**—Use as reagent a solution of titanium trichloride corresponding to 2 mg. of iron per cc. To a convenient volume of sample add a small excess of this reagent and compare with a standard solution of similar concentration prepared in the same way. The color is stable for about 30 minutes.

**Standard**—Prepare a standard solution to contain 1 mg. of tungsten per cc. by dissolving 1.358 gram of pure tungstic acid,  $\text{H}_2\text{WO}_4$ , or 1.261 gram of pure tungstic oxide,  $\text{WO}_3$ , in 100 cc. of 0.1 *N* or stronger sodium hydroxide solution and diluting to 1 liter. As a standard when results are to be in terms of tungstic oxide use 1.078 gram of tungstic acid or 1.000 gram of tungstic oxide.

#### TUNGSTEN AS THE COLLOIDAL OXIDE BY STANNOUS CHLORIDE

The method is similar to the preceding one but reduction is by stannous chloride. The reduction has also been reported with powdered lead.<sup>5</sup>

<sup>2a</sup> B. S. Hopkins, "Chemistry of the Rarer Elements," p. 268. D. C. Heath & Co., New York (1923).

<sup>3</sup> W. Singleton, *Ind. Chemist* 2, 454-7 (1926).

<sup>5</sup> A. Petrovskii, *J. Chem. Ind. (Moscow)* 7, 905-7 (1930).

**Sample**<sup>6</sup>—*Ore*. Use a 0.2 to 0.5 gram sample, according to concentration. Fuse with 4–5 grams of sodium peroxide in an iron crucible until decomposition is complete. When cool disintegrate with hot water, dilute and filter. Wash the residue with boiling water and concentrate to about 40 cc.

*Iron, Steel, Matte and Alloys*. Dissolve the sample in a mixture of 1 part of concentrated hydrochloric acid, 1 part of concentrated sulfuric acid and 3 parts of water. Evaporate just to dryness but do not ignite. Transfer to an iron crucible, add sodium peroxide and proceed as with ores.

*Films and Filaments*. Prepare as for the previous method.<sup>7</sup>

**Procedure**—The reagent consists of 10.3 grams of stannous chloride and 2.1 grams of stannic chloride dissolved in 100 cc. of 10 per cent acetic acid and 40 cc. of 85 per cent phosphoric acid. A precipitate may form which should be well dispersed before use.

To 40 cc. of sample solution add 6 cc. of the reagent and concentrated sulfuric acid to make 50 cc. Compare after 5 minutes with a standard solution<sup>8</sup> similarly treated at the same time.

#### TUNGSTEN BY POTASSIUM THIOCYANATE AND A REDUCING AGENT

Tungsten can be estimated by addition of a soluble thiocyanate and a reducing agent. Both stannous chloride and titanous chloride have been used as the reducing agent. In neutral solution an amethyst color is obtained. In strongly acid solution the color developed is green. By addition to alkaline solution as below, the color is yellow and suitable for colorimetric estimation.<sup>9</sup> The green color in acid solution has also been used.<sup>9a</sup>

Iron does not interfere up to 0.3 per cent, nor do phosphates up to 0.25 per cent. Molybdenum must be absent. Results are accurate to about 3 per cent in the range of 0.01 to 0.1 mg.

**Sample**—*Ores and Slags Free from Molybdenum and Vanadium*. Heat 0.1 gram of sample with 1 cc. of concentrated hydrofluoric acid and

<sup>6</sup> R. F. Heath, *Chem. Trade J.* 66, 629 (1920).

<sup>7</sup> See p. 376.

<sup>8</sup> See p. 376.

<sup>9</sup> F. Feigl and P. Krumholz, *Angew. Chem.* 45, 674-5 (1932).

<sup>9a</sup> F. S. Fer'yanchich, *Zavodskaya Lab.* 3, 301-3 (1934).

2 drops of 1:1 sulfuric acid. Fuse the residue with 0.5 gram of sodium carbonate and dissolve in hot water. Add a few drops of alcohol to reduce manganates and filter. Wash the filter and evaporate the combined filtrate and washings to dryness. Dissolve the salts in 2 cc. of water and use as sample.

*Alternative Method. Tungsten low.* When less than 0.02 per cent of tungsten is present, proceed as above but dissolve the sodium carbonate fusion in a large volume of water. Acidify to methyl orange with hydrochloric acid and add about 0.01 gram of iron as the chloride. Render the solution faintly alkaline with 1:1 ammonium hydroxide which will precipitate the iron and carry the tungsten down with it. Filter and ash. Fuse the ash with 0.5 gram of sodium carbonate and proceed as for the previous sample.

**Procedure—Reduction by Titanous Chloride.** Add 10 drops of 25 per cent potassium thiocyanate to a sample of about 2 cc. Dilute with water to 5 cc. and add about 4.5 cc. of concentrated hydrochloric acid. Boil 0.5 cc. of 15 per cent titanous chloride solution with 1 cc. of concentrated hydrochloric acid to expel traces of hydrogen sulfide and dilute to 10 cc. with 1:1 hydrochloric acid. Add 4 drops of this to the sample solution and dilute to 10 cc. with concentrated hydrochloric acid. Compare with a standard similarly treated.

*Reduction by Stannous Chloride.* Neutralize 2 cc. of the sample solution with 10 per cent sodium hydroxide solution and add excess to produce an alkalinity of 0.05–0.5 *N*. Add 5 drops of a 25 per cent solution of potassium thiocyanate. Dilute to 5 cc. Add 5 cc. of a 10 per cent solution of stannous chloride in concentrated hydrochloric acid, kept in a reduced condition with metallic tin. Mix and compare after 30 minutes with a standard similarly prepared.

#### TUNGSTEN BY HYDROQUINONE

Hydroquinone and tungstic acid dissolved in sulfuric acid give a red color.<sup>10</sup> Moisture dulls the color. Small amounts of alkalies, phosphates and nickel have no effect. Nitrate, iron, titanium, niobium, chromate and perrhenate interfere. Molybdenum in any considerable amount interferes. The method is accurate to 10 to 20 per cent.

**Sample**—Add to a slightly alkaline tungsten solution 0.5 cc. of a

<sup>10</sup> G. Heyne, *Z. angew. Chem.* 44, 237-8 (1931).

10 per cent solution of potassium hydroxide, and evaporate to dryness. Add 0.5 cc. of concentrated sulfuric acid and heat until sulfur trioxide fumes are evolved. At this stage the sample solution will be brown. Add a few crystals of potassium persulfate to decompose any organic matter. Heat until evolution of gas ceases. A yellow solution remains. Cool this in a desiccator.

**Procedure**—Add 1 cc. of a solution of 10 grams of hydroquinone in 100 cc. of concentrated sulfuric acid. The color becomes red. Dilute to a suitable volume with concentrated sulfuric acid and compare with a standard similarly treated.

**Standard**—Dissolve 63 mg. of pure tungstic oxide in 25 cc. of 10 per cent potassium hydroxide solution. Evaporate to dryness. Dissolve the residue in 10 cc. of concentrated sulfuric acid and dilute to 50 cc. with concentrated sulfuric acid. Each cc. contains 1 mg. of tungsten.

As standards for comparison dilute 0.1 cc. of this standard with 0.5 cc. of a solution of 16 grams of potassium sulfate in 90 cc. of concentrated sulfuric acid. Add 1 cc. of the hydroquinone reagent solution and dilute to a suitable volume with concentrated sulfuric acid. The standards must be protected from absorption of moisture.

#### TUNGSTEN BY RHODAMINE B

In dilute hydrochloric acid solution tungsten alters the yellowish red of a dilute Rhodamine B solution toward a violet. It is useful for estimation of tungsten in the absence of more than 10–20 times the amount of molybdenum.<sup>10</sup> Hydrochloric acid interferes if present in great excess. Sodium chloride and silicate do not interfere. The method is accurate to only 20 to 33 per cent. Comparison can be made only with a series of standards.

**Procedure**—Evaporate the acid sample to a small volume to free it from volatile acids. Neutralize and evaporate to 0.5 cc. Add 1 drop of concentrated hydrochloric acid. Add 2 cc. of a solution containing 0.1 gram of Rhodamine B per liter, and compare by both reflected and transmitted light with standards similarly treated at the same time.

The color is stable for 1 hour exposed to air. Violet flocks precipitate in a few days in the cold, or in a few minutes on heating.

## CHAPTER XXXIV

### MOLYBDENUM AND RHENIUM

#### MOLYBDENUM BY HYDROGEN PEROXIDE

SALTS of molybdic acid, in alkaline solution, may be oxidized with hydrogen peroxide to permolybdates, reddish brown in color.<sup>1</sup> As the intensity of the color is also proportional to the concentration of hydrogen peroxide, particular care must be taken to keep the latter constant. A variation in the amount of free alkali does not affect the color. The method is inapplicable in the presence of acids, or salts of chromium and tungsten. Ammonium salts must be decomposed by boiling with sodium hydroxide. In any case permolybdates are unstable so that the color is destroyed slowly on standing. A distinct reaction is obtained with 0.025 mg. of molybdic oxide using the volumes specified below.

**Sample—Molybdic Acid or Alkali Molybdates.** Dissolve a 1 gram sample in 10 cc. of 10 per cent sodium hydroxide solution. Dilute to 1 liter. As sample take 50 cc. in a Nessler tube.

**Ammonium Molybdate.** Dissolve a 1 gram sample in 10 cc. of 10 per cent sodium hydroxide solution. Add about 40 cc. of water and boil until the volume is reduced to about 25 cc. Dilute to 1 liter and take 50 cc. in a Nessler tube for the determination.

**Ferromolybdenum.** Treat 1 gram of finely powdered ferromolybdenum in a porcelain dish with 10 cc. of 1:1 nitric acid. Decant the nitric acid solution. Fuse the insoluble residue with 2 grams of a mixture of sodium and potassium carbonates. Dissolve in 25 cc. of water and add to the nitric acid solution. Heat to the complete removal of nitric oxide fumes. Add 10 cc. of 10 per cent sodium hydroxide solution, evaporate to dryness and ignite. When cool, take up with water and transfer to a 500 cc. flask. Dilute to volume. After complete sedimentation transfer a 50 cc. portion of the supernatant liquid to a Nessler tube.

**Tungsten and molybdenum deposits.**<sup>2</sup> Small deposits on the walls of

<sup>1</sup> A. D. Funck, *Z. anal. Chem.* 68, 283 (1926).

<sup>2</sup> W. Singleton, *Ind. Chemist* 2, 454-7 (1926).



electric bulbs are not conveniently handled by fusion methods. Dissolve such deposits in 3 per cent hydrogen peroxide rendered slightly alkaline with ammonium hydroxide or slightly acid with nitric acid. Deposits of the metals or their oxides are readily soluble in either. Transfer the solution to a beaker and evaporate nearly to dryness to decompose the peroxide. During evaporation add about 10 drops of 1:1 hydrochloric acid to prevent precipitation. Take up with distilled water and dilute to 50 cc.

*Tungsten and Molybdenum Wire.*<sup>2,4</sup> To a 4 gram sample in platinum add 30–40 cc. of strong hydrofluoric acid. Add concentrated or 1:1 nitric acid drop by drop, according to the violence of the reaction, until solution is complete. Evaporate nearly to dryness. Add 5 cc. of concentrated nitric acid and evaporate to dryness. Repeat this 3 times. Ignite at not over 550° to eliminate any organic matter and break up tungstic acid hydrates and complexes.

Dissolve in 2 cc. of 10 per cent sodium hydroxide solution. No residue of tungsten should remain. Filter, if necessary, on an inorganic filter. Wash the residue with 5 per cent sodium chloride solution. Dilute to 250 cc. and take a 50 cc. aliquot for the determination.

*Tungstic Acid.*<sup>4</sup> Treat a 5 gram sample with 5 cc. of concentrated nitric acid and evaporate to dryness. Repeat 3 times and proceed as above. Fusion with mixed carbonates will give low results for molybdenum.

**Procedure**—To sample and standards add 2 cc. of 3 per cent hydrogen peroxide. The color is partly discharged on dilution. If the color developed is not intense enough, increase the addition to sample and standards to 10 cc. of 3 per cent hydrogen peroxide or add 2 cc. of 30 per cent hydrogen peroxide solution.

**Standard**—Dissolve 1.5 gram of pure molybdenum trioxide in 10 cc. of 10 per cent sodium hydroxide solution. Dilute to 1 liter. Each cc. contains 1 mg. of molybdenum. Transfer 50 cc. to a Nessler tube for development of the color. If necessary dilute 100 cc. of the above to 1 liter to obtain a standard containing 0.1 mg. per cc.

#### MOLYBDENUM AS THE SULFIDE

Colloidal molybdenum sulfide varies according to concentration, from

<sup>4</sup> Walter J. King, *Ind. Eng. Chem.* 15, 350-4 (1923).

yellow to brownish red. Molybdenum in molybdates or oxides may be estimated by means of the color of the sulfide solution.<sup>7</sup> The method is exceedingly delicate, the difference in color between 0.01 and 0.02 mg. being easily perceptible by using a modified micro form of the method.<sup>8</sup> It has been applied to determination of the degree of decomposition of magnesium pyrophosphate in the usual method for phosphorus.

**Sample**—Weigh 0.1 gram of sample. Dissolve in 25 cc. of water and 1 cc. of 1:1 ammonium hydroxide. Boil off excess ammonia by concentrating to about 20 cc. If all the molybdenum is not in the hexavalent form pass in hydrogen sulfide until present in small excess. Bring to a boil to remove most of the excess of hydrogen sulfide. Add bromine-water to a faint coloration. Boil off excess bromine and cool. If any residue is undissolved add 1 cc. of 1:1 ammonium hydroxide and evaporate the excess by concentrating to 20 cc. Dilute to 500 cc. and pipet 25 cc. into a Nessler tube for development of color.

**Procedure**—Mix 5 cc. of glycerine with 100 cc. of water and saturate with hydrogen sulfide. The glycerine prevents subsequent precipitation of sulfur. To 25 cc. of sample in 1 tube and 25 cc. of standard in another, add 15 cc. of water and 5 cc. of the hydrogen sulfide solution. Add 0.2 N sulfuric acid drop by drop to each solution with constant stirring until the color of the more concentrated is no longer deepened. Dilute each to 50 cc. and compare by balancing.

**Standard**—Dissolve 0.1 gram of molybdenum trioxide in the same way as the sample. Dilute to 500 cc. The 25 cc. taken for development of color contains 5 mg. of molybdenum trioxide.

*Pure molybdenum trioxide.* If necessary to prepare pure molybdenum trioxide for use as a standard the following procedure may be used.<sup>7</sup> Pass hydrogen sulfide into a faintly ammoniacal solution of commercial ammonium molybdate. Acidify with 1:1 sulfuric acid. Filter the precipitate on a fine-grained filter and wash with hot water until free from sulfates. Dry, separate from the filter and roast in a porcelain crucible until the white molybdenum trioxide is obtained. Keep in a desiccator. For standardization 1.5 grams of the trioxide is equivalent to 1 gram of molybdenum.

<sup>7</sup> E. Wendehorst, *Z. anorg. Chem.* 144, 319-20 (1925).

<sup>8</sup> J. M. McCandless and J. I. Burton, *Ind. Chem. Eng.* 19, 406 (1927).

## MOLYBDENUM BY POTASSIUM XANTHATE

Molybdenum may be determined by the red color of the xanthate, the colored compound being extracted with a mixture of petroleum and ethyl ethers or with chloroform before comparison.<sup>10,10a,2</sup> The method is accurate to about 8 per cent.

**Sample**—Prepare as for the hydrogen peroxide method.<sup>12</sup>

**Reagent**—Prepare 100 cc. of a saturated solution of potassium hydroxide in absolute alcohol. Shake with carbon disulfide in excess until no further reaction occurs. Leave a small amount of carbon disulfide in the bottom to insure saturation.

**Procedure**—To the solution of sample add 0.2 *N* sodium hydroxide solution to faint alkalinity. Add 5 cc. of reagent, mix well and add 30 per cent acetic acid until distinctly acid. Extract with 25 cc. of a mixture of 35 per cent petroleum ether and 65 per cent ethyl ether. Repeat with another 25 cc. portion of solvent. Mix the two colored extracts and compare, by dilution with solvent, with a standard prepared from molybdenum trioxide solution<sup>13</sup> by similar treatment. Chloroform may also be used for extraction of the color.

## MOLYBDENUM BY POTASSIUM THIOCYANATE

The amber to reddish brown color of molybdenum thiocyanate has been used for the determination of molybdenum in steel<sup>14,15</sup> where many rapid determinations are to be made. The colored compound is soluble in ether, butyl acetate,<sup>16</sup> or cyclo-hexanol,<sup>16a</sup> which may therefore be used for extraction of the color. The method has also been used for the determination of small quantities of molybdenum in tungsten.<sup>4</sup> King found no interference by the metals commonly associated with tungsten when the color is extracted with ether. In steel the method is best for amounts below 1 per

<sup>10</sup> S. L. Malowan, *Z. anorg. Chem.* 108, 73-80 (1919).

<sup>10a</sup> D. Hall, *J. Am. Chem. Soc.* 44, 1462 (1922).

<sup>12</sup> See pp. 380-1.

<sup>13</sup> See p. 382.

<sup>14</sup> O. L. Maag and C. H. McCollam, *Ind. Eng. Chem.* 17, 524 (1925).

<sup>15</sup> "Methods of Chemists of the U. S. Steel Corporation for Sampling and Analysis of Alloy Steels," 2nd ed., p. 72 (1921).

<sup>16</sup> L. H. James, *Ind. Eng. Chem., Anal. Ed.* 4, 89-90 (1932).

<sup>16a</sup> Loren C. Hurd and Fred Reynolds, *Ind. Eng. Chem., Anal. Ed.* 6, 477-8 (1934).

cent, but has been successfully applied with as high as 2.5 per cent of molybdenum present.<sup>18</sup> At more than 0.8 per cent of molybdenum it is not as accurate as the sulfide or lead molybdate methods. Iron, chromium, nickel, vanadium, silicon, and tungsten do not interfere. If more than 0.13 per cent of copper is present, copper thiocyanate must be removed. For samples containing only a minor amount of molybdenum it can be removed from solution by adsorption on manganese dioxide, which is then dissolved and used as sample.<sup>19,20</sup>

The variations between the minimum and maximum values found was less than 10 per cent in work on tungsten. The colorimetric method for molybdenum in tungsten is advisable between 10 and 300 p.p.m. Properly applied the method will detect 0.001 mg. of molybdenum.<sup>20a,20b</sup> Zinc in hydrochloric acid was originally used<sup>21</sup> for reduction of any ferric ion present. Stannous chloride is a more convenient reducing agent. Sodium thiosulfate may also be used.<sup>21a</sup>

**Sample for Extraction—Tungsten and Tungstic Acid.** Follow the procedure under the peroxide method<sup>22</sup> and complete as follows. Neutralize the sample solution or an aliquot with 1:10 hydrochloric acid, using litmus as an indicator. Avoid precipitation of tungstic acid. As soon as the solution is acid add 20 cc. of 60 per cent tartaric acid solution for every 6 grams of tungstic oxide or fraction thereof. Add 25 cc. of 1:5 hydrochloric acid.

**Steel.** Dissolve 0.5 gram of steel filings in 10 cc. of an acid mixture consisting of 225 cc. of concentrated sulfuric acid, 350 cc. of concentrated nitric acid and 750 cc. of water. Evaporate to copious fumes. Avoid spattering without covering the beaker, by regulating the heat. All nitrates must be removed. Cool and add 30 cc. of a second acid mixture consisting of 100 cc. of concentrated hydrochloric acid, 450 cc. of concentrated sulfuric acid and 1450 cc. of water. Boil until all salts are dissolved. An exact amount of hydrochloric acid is important. Too

<sup>18</sup> Thomas R. Cunningham and H. L. Hamner, *Ind. Eng. Chem., Anal. Ed.* 3, 106-7 (1931).

<sup>19</sup> Bartholow Park, *Ind. Eng. Chem., Anal. Ed.* 6, 189-90 (1934).

<sup>20</sup> See p. 214.

<sup>20a</sup> J. S. Böhm and J. Vostrebal, *Z. anorg. allgem. Chem.* 110, 81 (1920).

<sup>20b</sup> N. A. Tananaev and G. E. Panchenko, *Ukrain. Khim. Zhur.* 4, Sci. Pt. 121 (1929).

<sup>21</sup> A. D. Braun, *Z. anal. Chem.* 6, 86 (1867).

<sup>21a</sup> Kenneth E. Stanfield, *Ind. Eng. Chem., Anal. Ed.* 7, 273-4 (1935).

<sup>22</sup> See pp. 380-1.

much results in rapid fading of the color before it can be extracted. A deficiency gives a difference in quality of the color developed.

*Steel. Alternative Method.*<sup>18</sup> Dissolve 0.5 gram of steel, or 1 gram if less than 0.1 per cent of molybdenum is present, in 25 cc. of 1:4 sulfuric acid at 60°. When the reaction is complete add 3 cc. of 30 per cent hydrogen peroxide. Boil for 5 minutes to remove hydrocarbons and at least partially reduce the molybdenum. Filter on a small filter paper and wash. Discard the residue of carbon. Evaporate the filtrate and washings to about 25 cc. to decompose excess of hydrogen peroxide. If more than a trace of tungsten is present, add 1 gram of citric or tartaric acid to prevent precipitation of tungsten. Render slightly alkaline with 10 per cent sodium hydroxide solution. Add 1:1 sulfuric acid until distinctly acid.

*Steel or Iron. Alternative Method.*<sup>18</sup> Dissolve a 0.1 gram sample in 5 cc. of 1:2 nitric acid and 5 cc. of 60 per cent perchloric acid. During the heating keep the beaker covered with a raised watch glass. For stainless steel also add 10 cc. of 1:1 hydrochloric acid. Boil for 10 minutes after copious fumes are evolved. Cool and dissolve in 15 cc. of water.

*Plants.*<sup>24a</sup> Moisten 100 grams of air-dried sample with concentrated sulfuric acid, heat to drive off excess acid, and ignite in a muffle <sup>24b</sup> below 550° with occasional stirring. Extract the ash with 20 cc. of 1:4 hydrochloric acid and filter. Ignite the residue, which still contains some carbon, and extract this ash with 10 cc. of 1:4 hydrochloric acid. Filter and digest the residue with 10 cc. or more of *aqua regia*. Dilute with about 30 cc. of water and filter. Wash thoroughly and discard the colorless silicious residue. Evaporate the combined filtrates and washings to dryness to remove nitric and excess hydrochloric acids. Take up the residue with 10 cc. of 1:10 hydrochloric acid.

If tungsten is believed to be present <sup>24c</sup> add 5 grams of tartaric acid and 1:1 ammonium hydroxide until the solution is strongly alkaline. While cold saturate with hydrogen sulfide. Pour the solution into an excess of 6 *N* hydrochloric acid to precipitate molybdenum trisulfide and heat to boiling to coagulate the precipitate. If not much calcium is present 6 *N* sulfuric acid may be substituted. If vanadium is present but not tungsten precipitate the molybdenum sulfide from the acid solution of the salts from the ash and heat in a water bath under pressure for 30 minutes.

<sup>24a</sup> Kenneth E. Stanfield, *Ind. Eng. Chem., Anal. Ed.* 7, 273-4 (1935).

<sup>24b</sup> P. H. M.-P. Brinton and A. E. Stoppel, *J. Am. Chem. Soc.* 46, 2454 (1924).

<sup>24c</sup> H. A. Doerner, *Bur. Mines Circ.* 6079 (1928).

Filter the precipitated sulfide and wash with a cold saturated solution of hydrogen sulfide. Dissolve by pouring through the filter a warm mixture of equal parts of 6 *N* hydrochloric acid and concentrated nitric acid. Evaporate this extract to dryness. To oxidize the molybdenum to the hexavalent condition add 2 drops of concentrated nitric acid and again evaporate to dryness. Take up with 5 cc. of water and 0.5 cc. of 6 *N* hydrochloric acid.

*Soil.*<sup>24a</sup> Cover 200 grams of soil with 1:4 hydrochloric acid and warm on a steam bath for 24 hours. Filter and wash the residue. Repeatedly treat the residue with a mixture of concentrated hydrochloric and nitric acids until only a colorless residue is left. Evaporate the combined extracts and complete as for plants. Because of the large amounts of substances present a reprecipitation of molybdenum sulfide is desirable to get adequate concentration.

**Procedure by Extraction—*Tungsten or Steel.*** Dilute the solution to about 100 cc. Add 10 cc. of 5 per cent potassium thiocyanate solution for steel samples or 5 cc. of 30 per cent solution for tungsten samples. Cool to room temperature. Mix well and add 10 cc. of a solution containing 250 grams of stannous chloride dissolved in 200 cc. of concentrated hydrochloric acid by boiling, and diluted with 800 cc. of water. Keep the stannous chloride solution over metallic tin to prevent oxidation. Mix well. All the iron should be reduced before the ether extraction is made. The molybdenum is also reduced to the pentavalent or tetravalent state. The thiocyanate forms a complex potassium-molybdenum thiocyanate.

Transfer the solution to a separatory funnel, cool below room temperature and shake with 10 cc. of ether or cyclohexanol. Return the acid solution to the beaker and draw the ether into a 50 cc. comparison cylinder. Extract the aqueous portion successively with 10 cc. portions of solvent until the extracts are colorless. Iron, chromium, nickel and tungsten remain in the acid layer. A single extraction with 20 cc. of butyl acetate at this stage, followed by washing the extract with the reagent, is stated to give removal of molybdenum within the accuracy of the method.<sup>16</sup>

Let the extract stand for 5 minutes protected from evaporation, and compare with a standard by dilution or balancing. A special colorimeter has been designed for the purpose. Avoid heating the solutions with artificial light. The completeness of five successive extractions of color with ether has been reported as 85.7, 95.4, 98.4, 99.7 and 100.

*Plants or Soil.* To the sample or an aliquot containing 0.5 cc. of 6 *N* hydrochloric acid add 1 cc. of 10 per cent potassium thiocyanate solution and 2 cc. of 20 per cent stannous chloride solution. Extract at once with butyl acetate previously saturated with stannous chloride and potassium thiocyanate solutions. For 0.001 mg. of molybdenum use 5 cc., for 10 mg. use 25 cc. Use half for the first extraction and divide the balance for 3 more extractions. Combine the extracts and if turbid add a few drops of alcohol. Compare at once with the color developed from a suitable amount of ammonium molybdate solution in the same way at the same time. Add 0.5 cc. of 6 *N* hydrochloric acid to the standard as the rate of fading of the color is directly affected by the acidity of the solution.

**Standard—Steel.** Treat two 0.5 gram samples of steel having a known molybdenum content in exactly the same manner as the unknown. Dilute each extract to 50 cc. and see that they match in color. If the two match, dilute one to 100 cc. for a low molybdenum steel, or combine the two for a high molybdenum steel. Ether standards must be renewed every two hours<sup>14</sup> due to evaporation. If this can be prevented the color is stable for at least 7 days.<sup>18</sup>

*Alternative Steel Standard.*<sup>18</sup> Dissolve 0.430 gram of pure sodium molybdate in 1 liter of water containing 10 cc. of 1:1 sulfuric acid. Mix carefully and transfer 100 cc. to a beaker. Add 12 cc. of 1:1 sulfuric acid and pass the solution through a Jones zinc reductor into 35 cc. of a solution containing 25 grams of ferric sulfate in 950 cc. of water, 40 cc. of syrupy phosphoric acid and 10 cc. of concentrated sulfuric acid. Titrate with 0.05 *N* potassium permanganate solution. Each cc. is equivalent to 0.0016 gram of molybdenum. Run a blank on this standardization by passing 100 cc. of water and 12 cc. of 1:1 sulfuric acid through the reductor into 35 cc. of the iron solution. This blank will usually amount to about 0.2 cc. of 0.05 *N* permanganate solution. Calculate the strength of this standard molybdenum solution.

Estimate the amount of molybdenum required by the intensity of color in the sample. Add the required amount of standard to 25 cc. of 8 per cent ferric sulfate solution in 20 per cent sulfuric acid. Develop the color according to the procedure by extraction.<sup>29</sup>

*Ammonium Molybdate Standard.* Dissolve 0.2042 gram of ammonium molybdate in water and dilute to 1 liter. Each cc. contains 0.1 mg. of molybdenum. For further use dilute 10 cc. to 100 cc. giving 0.01 mg. per cc., and 10 cc. to 1 liter giving 0.001 mg. per cc.

<sup>29</sup> See p. 386.

**Sample without Extraction**—A somewhat different method may be followed, using the same color reaction, but omitting the extraction with solvent.<sup>30</sup> Vanadium if present must be separated by a method given below.

*Steel.* Dissolve 1 gram of steel in 50 cc. of 1:2 hydrochloric acid containing 60 grams per liter of disodium phosphate. Heat until the evolution of hydrogen is complete. Add 25 cc. of a 5 per cent solution of potassium chlorate and warm 1 or 2 minutes, covered. Wash off the cover glass, dilute with 100 cc. of hot water and boil 3 minutes to drive off excess chlorine. Neutralize with 10 per cent sodium hydroxide solution while hot, to the appearance of a faint precipitate of iron phosphate. Redissolve the precipitate with a few drops of concentrated hydrochloric acid. If the steel contains vanadium add a solution of ferrous chloride equivalent to 1 gram of ferrous oxide, and boil for a few seconds. Vanadium is reduced from the pentoxide to the tetroxide and precipitated quantitatively.

Pour the solution slowly into hot 16 per cent sodium hydroxide solution in a 500 cc. volumetric flask, using 100 cc. of the sodium hydroxide solution with vanadium-free steel, and 120 cc. in the case of a steel freed from vanadium. Shake, cool quickly, dilute to volume, and filter through double filter papers. Use 20 cc. of the clear filtrate for comparison.

**Procedure without Extraction**—Treat 20 cc. of the unknown and 20 cc. of a standard solution of properly diluted sodium molybdate with 10 cc. of a 10 per cent solution of potassium thiocyanate. As a stannous chloride solution dissolve 30 grams of stannous chloride in 150 cc. of hot concentrated hydrochloric acid and dilute with 1 liter of 1:4 hydrochloric acid. Add 10 cc. of stannous chloride solution to standard and sample and compare at once by balancing.

The method is reported as taking from 25 to 30 minutes, with an accuracy of about 1.5 per cent up to 5 per cent of molybdenum. With a higher content of molybdenum use less filtrate, and dilute to 20 cc. with a 2.5 per cent sodium hydroxide solution. A moderate excess of free acid does no harm but must be nearly the same in sample and standard as the rate of fading increases with increased acid concentration.

**Standard Sodium Molybdate Solution**—Dissolve 25 grams of sodium hydroxide in about 200 cc. of water and add 0.0375 gram of pure molybdic oxide. Heat until dissolved and pour into a 250 cc. volumetric

<sup>30</sup> J. Kassler, *Chem.-Ztg.* 51, 953 (1927).



flask. Dilute to volume. Each cc. corresponds to 0.1 mg. of molybdenum. In using varying amounts of this solution, dilute each to 20 cc. with a 2.5 per cent sodium hydroxide solution to keep the concentration of free alkali constant.

#### MOLYBDENUM BY PHENYLHYDRAZINE

A saturated aqueous solution of phenylhydrazine, to which mineral acid has been added, gives a pink to blood red color on warming with molybdenum solutions.<sup>31</sup> Iodates give free iodine, persulfates a faint yellow, vanadates green; permanganates are decolorized. Perchlorates, perborates and tungstates are without effect. The solution must be acid to avoid precipitation. Apparently the reaction is specific for molybdenum. The color is stable for several hours and will detect 0.02 mg. of molybdenum. Results agree well with the thiocyanate method.

**Sample** <sup>32</sup>—*Manganese-iron Ore*. Dissolve 1 gram of finely powdered ore in 40 cc. of 1:1 hydrochloric acid. Boil gently until evolution of chlorine ceases. Filter and wash the filter with hot water. Evaporate the filtrate to dryness to eliminate excess hydrochloric acid. Take up the residue with 30 cc. of 1:100 hydrochloric acid. Precipitate manganese and iron by adding the solution to 20 cc. of hot 16 per cent sodium hydroxide solution in a 100 cc. flask. Filter through a dry filter and use an aliquot of the filtrate for the determination.

**Procedure**—To 10 cc. of sample solution add 5 cc. of a reagent containing 3 parts of phenylhydrazine and 3 parts of sulfuric acid to 65 parts of water. Heat on a water bath to 80° for 15 minutes and let cool for 30 minutes. If the original solution was alkaline, add 5 cc. of 10 per cent sulfuric acid. Compare with a standard <sup>32a</sup> similarly developed.

#### MOLYBDENUM BY TANNIC ACID

Addition of dilute tannic acid solution to an acetic acid solution of molybdenum gives a color suitable for estimation of the molybdenum content.<sup>33</sup> Not over 2 per cent of molybdenum should be present if a

<sup>31</sup> E. Montignie, *Bull. soc. chim.* [4] 47, 128 (1930).

<sup>32</sup> H. Hauptmann and M. Baleoni, *Z. anorg. allgem. Chem.* 214, 380-4 (1933).

<sup>32a</sup> See p. 387.

<sup>33</sup> G. Spurge, *Chem. Eng. Mining Rev.* 11, 253 (1919).

1 gram sample is used. The method has been successfully applied to aqueous solutions containing arsenic and bismuth.<sup>34</sup>

**Sample**—Dissolve a 1 gram sample in 10 cc. of concentrated nitric acid. Heat gently for 30 minutes and evaporate to dryness on a water bath. Add 40 cc. of 1:3 hydrochloric acid and warm. If tungsten is present, filter. Add 15 cc. of concentrated ammonium hydroxide and boil for 5 minutes. Filter at once into a 250 cc. volumetric flask and wash the filter with hot water. Do not dilute to volume at this time.

Rinse the precipitate back into the original flask or beaker, dissolve in 10 cc. of concentrated hydrochloric acid and precipitate with 15 cc. of concentrated ammonium hydroxide. Filter into the volumetric flask with the first molybdenum solution and wash the precipitate well. Render the filtrate acid with acetic acid and add 10 cc. of glacial acetic acid in excess. Cool, dilute to volume and mix well.

By neutralizing, then acidifying with acetic acid, samples prepared according to other methods should be suitable.

**Procedure**—Put 2 cc. of fresh 0.5 per cent tannic acid in each of two 50 cc. Nessler tubes. To one add 50 cc. of sample and mix well. To the other add the prepared standard with suitable amounts of distilled water until the color and volume of the sample is duplicated. The amount of standard required should be not less than 40 cc. owing to the effect of dissolved salts and excess acid.

**Standard**—Dissolve 9 grams of crystallized ammonium molybdate,  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ , in water and dilute to 1 liter. Standardize by precipitation of a definite volume with lead as lead molybdate and convert the precipitate so obtained to lead sulfate. Every 0.2106 gram of lead sulfate is equivalent to 0.1 gram of molybdic oxide. Dilute to a concentration where each cc. contains 0.10 mg. of molybdic oxide.

Measure a suitable volume, such as 10 cc., into a flask. Add 20 cc. of concentrated hydrochloric acid, dilute to about 100 cc. and add 30 cc. of concentrated ammonium hydroxide. Boil for 5 minutes. When cool transfer to a 250 cc. volumetric flask and neutralize with acetic acid. Add 10 cc. of excess glacial acetic acid, dilute to volume and mix well.

#### MOLYBDENUM BY A TUNGSTIC ACID REAGENT

The reducing action of stannous chloride on molybdenum and tung-

sten in hydrochloric and phosphoric acids causes a light blue to deep violet color proportional to the molybdenum content.<sup>35</sup> The reaction is suitable for estimation of traces of molybdenum in steel. Vanadium must be absent. It is conveniently removed by reducing the vanadium and part of the iron with sodium bisulfite and precipitating with sodium hydroxide. Tungsten may be eliminated by oxidation with chlorate and filtration from insoluble tungstic acid. The method will detect 1 part in 8 million.

**Sample—Steel.** Dissolve a suitable sample in 10 cc. of 1:1 hydrochloric acid. To the hot solution add a few crystals of potassium chlorate to oxidize tungsten to tungstic acid. Heat to incipient crystallization and dilute to 10 cc. with distilled water. Heat 25 cc. of 5 per cent sodium hydroxide solution to boiling. Slowly pour the solution of sample into this in a fine stream while the solution is boiling actively. Molybdenum is in solution. When cool dilute to 50 cc. and filter on a dry filter. Neutralize an aliquot of the filtrate for use as sample. For very accurate work allowance must be made for a small amount of molybdenum retained by the tungstic acid precipitate.

**Reagent—**Dissolve 11.4 grams of ammonium tungstate containing 70 per cent of tungsten, in 40 cc. of 10 per cent sodium hydroxide solution. Heat if necessary. Add 70 cc. of 30 per cent tartaric acid solution and 5 cc. of concentrated hydrochloric acid. Dilute to 500 cc. and pass hydrogen sulfide through the solution for 30 minutes. After standing 12 hours filter out molybdenum sulfide originally present as molybdate in the tungsten and wash the precipitate. Boil off the hydrogen sulfide from the solution and when cool dilute to 2 liters.

**Procedure—**Dilute the sample to about 50 cc. Add 6 cc. of 1:1 hydrochloric acid, 1 cc. of syrupy phosphoric acid and 5 cc. of the tungstic acid reagent. Dilute to about 80 cc. and add 10 cc. of a stannous chloride solution, produced by dissolving 20 grams of tin in 200 cc. of concentrated hydrochloric acid and diluting to 1 liter. Dilute to 100 cc. Compare the color which develops at once, with that produced by similar treatment of a suitable standard sodium or ammonium molybdate solution.<sup>36</sup> The color is very stable.

<sup>35</sup> Ernest Bertrand, *Chimie et Industrie* 198, Special No. March, 1931.

<sup>36</sup> See p. 386.

## BY SODIUM

When excess of sodium thiosulfate solution is added to a molybdate solution acidified with hydrochloric acid a precipitate of sulfur separates and the molybdenum is reduced to give a color extractable with ether or ethyl acetate. The color in the organic solvent is suited to colorimetric estimation against a series of standards.<sup>37</sup> The color varies with concentration from lilac to rose to red-brown. Five mg. of molybdic acid give a red color, 0.005 mg. a lilac. The reaction is more sensitive than those with stannous chloride, hydrogen peroxide or potassium xanthate. Alkali destroys the color. The color is not affected by tartaric, citric, oxalic or tannic acids, ammonium salts, chromates, chlorates, and many cations. Copper and iron in sufficient concentration may interfere. The details of color production are fairly well developed but published applications are limited.

**Sample**—*Steel*.<sup>38</sup> Dissolve 1–2 grams in *aqua regia* and evaporate to dryness. Redissolve in 10 cc. of concentrated hydrochloric acid and dilute with 20–30 cc. of water. Filter and dilute to 100 cc.

**Procedure**—Add 2 cc. of concentrated hydrochloric acid to 10 cc. of the sample solution and extract with 8 cc. of a mixture of 80 per cent ethyl acetate and 20 per cent ether. Shake the solvent layer with an equal volume of 30 per cent sodium thiosulfate solution. Separate the layers and repeat with fresh thiosulfate solution.

Filter the solvent layer and compare with a series developed from steels with known amounts of molybdenum present or added.

## RHENIUM BY POTASSIUM THIOCYANATE

Rhenium reacts like molybdenum with thiocyanate to give a yellow to red color. This can be extracted with ether or ethyl acetate and used for quantitative estimation.<sup>39</sup> Nickel, manganese, chromium, gold and platinum do not interfere. Addition of stannous chloride to reduce the iron present prevents its interference. Other substances reacting with thiocyanate or stannous chloride to give colored products must be absent. Acidity, time, temperature and concentration of stannous chloride must

<sup>37</sup> Pietro Falciola, *Ann. chim. applicata* 17, 261-2 (1927).

<sup>38</sup> L. Losana and M. Jarach, *Industria Chimica* 9, 623-5 (1934).

<sup>39</sup> W. Geilmann, F. W. Wrigge and F. Weibke, *Z. anorg. allgem. Chem.* 208, 217-24 (1932).

be standardized. Of particular importance is the time intervening between addition of stannous chloride and extraction of color with the solvent. Not more than 2 mg. of rhenium per 50 cc. of extract should be present. By use as a micro method the procedure will detect 0.001 mg. of rhenium.

**Procedure**—Transfer 25 cc. of nearly neutral perrhenate solution to a separatory funnel. In another take 25 cc. of a standard of very nearly the same concentration. To each add 10 cc. of 1:1 hydrochloric acid and 2 cc. of 10 per cent potassium thiocyanate solution. Dilute each to 50 cc. and add 10 cc. of 2 per cent stannous chloride solution. Shake at once. At the end of exactly 30 seconds add 20 cc. of ether or ethyl acetate and shake. This solvent should have previously been shaken with a mixture of 20 cc. of 2 per cent stannous chloride solution, 5 cc. of 10 per cent potassium thiocyanate solution, 10 cc. of 1:1 hydrochloric acid and 20 cc. of water, to remove peroxides. Separate the solvent layers and repeat the extractions with two 10 cc. portions for each. Combine the extracts from the sample and those from the standard. Dilute each to 50 cc. with solvent and compare by balancing.

**Standard**—Prepare a solution of potassium perrhenate to contain 0.1 mg. of rhenium per cc. Use suitable amounts of this diluted to 25 cc. as standards.

## CHAPTER XXXV

### URANIUM

#### URANIUM BY *o*-HYDROXYBENZOIC ACID

THE red color of a solution of the salt formed by the reaction of *o*-hydroxybenzoic acid and a uranyl salt may be used for the determination of the latter.<sup>1</sup> Iron and organic solvents such as alcohol and acetone must be absent. Neutral salts do not interfere. Mineral acids or large amounts of organic acids must also be absent. The method is accurate to about 7 per cent.

**Sample**—Uranium should be in the form of the nitrate. If free mineral acid or considerable amounts of organic acids are present, add sodium acetate in excess of the free acidity and boil until substantially all of the acetic acid has been driven off.

**Procedure**—To 25 cc. of the solution containing uranium in the form of uranyl nitrate add 25 cc. of a 2 per cent solution of the sodium salt of *o*-hydroxybenzoic acid. At the same time similarly treat 25 cc. of standard or a suitable amount of standard diluted to 25 cc. Compare by balancing.

**Standard Solution**—Prepare a 2 per cent aqueous solution of uranyl nitrate and determine the uranium content gravimetrically. Dilute so that 1 cc. corresponds to 0.1 mg. of uranium.

#### URANIUM BY PHENOLIC ACIDS

Phenolic acids such as tannic, gallic and resorcylic acids give a brown coloration with dilute uranium solutions. Sodium acetate intensifies this. Prior addition of sodium acetate gives a less intense color than addition after the color has been first developed by the phenolic acid.<sup>2</sup> Ammonium chloride, sodium phosphate, potassium pyrosulfate, Rochelle salt, am-

---

<sup>1</sup> Müller, *Chem.-Ztg.* 43, 739-40 (1919).

<sup>2</sup> Pabitra Nath Das-Gupta, *J. Indian Chem. Soc.* 6, 763-76 (1929).

monium fluoride and other acid salts must be absent. Excess of acetic, hydrochloric or nitric acid must be evaporated or neutralized. Tannic acid gives the most sensitive reaction. Sodium acetate may cause the tannic acid color to become colloidal, in which case gallic acid should be used.

**Procedure**—To 25 cc. of sample add 2 cc. of fresh 1 per cent tannic acid solution. Mix well and add 3 cc. of 5 per cent sodium acetate solution. Dilute to 50 cc. with water and mix well. Compare at once with a standard prepared simultaneously, using the balancing method. The colors are affected by standing, exposed to the air.

#### URANIUM BY POTASSIUM FERROCYANIDE

The reaction of uranium with potassium ferrocyanide to give a red color may be used for the determination of uranium.<sup>3</sup> Other metals must be separated, with the exception of aluminum.<sup>4</sup> The method is suitable for samples of material containing 0.4 to 1.2 per cent uranium as uranium oxide,  $U_3O_8$ .

**Sample**—*Organic Matter*. The amount of sample taken should correspond to 2 to 6 mg. of uranium oxide,  $U_3O_8$ . Ash the sample by any convenient method. Dissolve the ash in a mixture of hydrochloric and nitric acids. If much calcium is present, add sulfuric acid to precipitate calcium as sulfate. Filter and wash the precipitate. Separate uranium from the filtrate as given in detail for ores.

*Low Grade Uranium Ore*. Weigh 0.5 gram of ore. If the ore contains much carbonate, wet the sample first with 5 cc. of water. Add 20 cc. of 1:4 sulfuric acid with care to prevent loss from spattering. Add 5 cc. of 1:2 hydrochloric acid and boil gently for one-half hour. Dilute the solution to 50 cc. with hot water and filter. Wash the residue with hot 1:4 sulfuric acid.

To the filtrate and washings add carbonate-free ammonium hydroxide and 4 to 5 cc. of 3 per cent hydrogen peroxide solution. This precipitates the metals as sesquioxides. Filter and wash the precipitate with a hot 3 per cent solution of ammonium sulfate containing a few drops of concentrated ammonium hydroxide. Dissolve the precipitate in the smallest

<sup>3</sup> Arturo Bruttini, *Gazz. chim. ital.* 23, 251-7 (1893).

<sup>4</sup> J. Tschernichow and E. Guldina, *Z. anal. Chem.* 96, 257-63 (1934).

possible volume of hot 1 per cent sulfuric acid. The total volume should not be over 50 cc.

Transfer this solution, which now contains uranium, iron, aluminum and possibly vanadium, to an electrolysis vessel for the separation of iron. Electrolyze, using a mercury cathode, with a current of 4 to 5 amperes and 6 to 8 volts. Test for iron in the solution with a 0.2 per cent solution of potassium ferricyanide on a spot plate. When the solution is free from iron, transfer to a beaker, rinsing out the electrolysis chamber. The total volume of solution should not be over 100 cc. at this point.

Precipitate uranium and aluminum as before, with ammonium hydroxide in the presence of hydrogen peroxide. If vanadium is present, uranium is precipitated mostly as uranium vanadate. Wash the precipitate 3 or 4 times with a hot 3 per cent ammonium sulfate solution containing a few drops of concentrated ammonium hydroxide. If the ore contains no vanadium, dissolve the ammonium uranate precipitate in 0.2 per cent sulfuric acid. Test the solution for iron. If free from iron, make up to 100 cc. with the same acid.

If vanadium is present, dissolve the precipitate in 3 per cent sulfuric acid. Neutralize with 1:2 ammonium hydroxide until a permanent turbidity remains. Remove the turbidity by addition of a few drops of *N* sulfuric acid. Be careful to avoid using any more acid than is necessary to make the solution clear. Dilute to about 40 cc.

Uranium in the sulfuric acid solution is precipitated as phosphate, leaving the vanadium in solution. To the sulfuric acid solution add 5 cc. of 1:2 acetic acid and 15 cc. of 0.33 *M* disodium phosphate solution. If the amount of aluminum present is very small, coagulation is improved by the addition of 2 cc. of an alum solution containing 5 mg. of aluminum oxide per cc. Aluminum helps carry down uranium phosphate. Heat to boiling and let stand for 10 minutes. Filter and wash 5 or 6 times with *N* ammonium nitrate solution. Vanadium is completely removed in the filtrate and washings. Dissolve the uranium phosphate precipitate in 25 cc. of hot 0.2 per cent sulfuric acid. Make up to 100 cc. with the same acid.

**Procedure—Balancing.** To 10 cc. of prepared sample solution, and to 10 cc. of a standard solution corresponding to 0.1 mg. of uranium oxide,  $U_3O_8$ , per cc., add 5 cc. of a 10 per cent solution of potassium ferrocyanide containing 1 per cent of sodium sulfite. Compare in a colorimeter after 3 or 4 minutes.

**Series of Standards.** The sample solution may be compared with a series of standards. Into a series of tubes put 1, 2, 3, 4, 5, 6 and 7 cc.



respectively of the standard solution containing 0.1 mg. uranium oxide per cc. Bring the volume of each to 10 cc. with 0.2 per cent sulfuric acid. Add to each 5 cc. of potassium ferrocyanide reagent as described above. Compare with 10 cc. portions of sample solution similarly treated. Intermediate standard tubes may be prepared if necessary in order to approach the color of the sample as closely as possible.

**Standard**—Dissolve 1.7885 grams of iron-free uranyl nitrate in water and dilute to 1 liter. This corresponds to about 1 mg. of uranium oxide,  $U_3O_8$ , per cc. The concentration of uranium should be determined exactly by gravimetric analysis. Precipitate uranium from 50 cc. of this solution with carbonate-free ammonium hydroxide in the presence of 4 to 5 cc. of 3 per cent hydrogen peroxide solution. Wash the precipitate with 3 per cent ammonium sulfate solution containing a few drops of ammonium hydroxide. Dissolve the ammonium uranate precipitate in 2 per cent sulfuric acid and dilute to 500 cc. with the same acid. This solution corresponds to about 0.1 mg. of uranium oxide,  $U_3O_8$ , per cc., the exact value having been shown by analysis.

## CHAPTER XXXVI

### BERYLLIUM

#### BERYLLIUM BY CURCUMIN

IN FAINTLY alkaline solution a trace of beryllium is precipitated as the hydroxide and adsorbs curcumin to give an orange-red color.<sup>1</sup> A solution containing 50 mg. of beryllium per liter gives a red flocculent precipitate. The method is suitable for quantitative estimation in concentrations from 0.05 to 1 mg. per liter. A blank solution is yellow to brown in color. Potassium, sodium, lithium, calcium and barium do not interfere. Magnesium decreases the sensitivity of the reaction, although 1 mg. of beryllium may be detected in the presence of 1 gram of magnesium per liter. Aluminum and iron interfere but can be removed. Sodium fluoride decreases the sensitivity of the reaction.

**Sample**—Dissolve the sample by a suitable method according to its source. To remove aluminum acidify the solution slightly with hydrochloric acid, and treat with excess sodium fluoride. After 1 hour filter off the precipitate of sodium aluminofluoride,  $\text{Na}_3\text{AlF}_6$ . The amount of aluminum remaining in solution is not sufficient to interfere. Iron is removed at the same time. Iron may also be removed by adding excess sodium hydroxide solution, in which beryllium is soluble due to its amphoteric properties. Another method of separation is by precipitation of aluminum, iron and zirconium by 8-hydroxyquinoline in an acetic acid-ammonium acetate buffered solution.

**Procedure**—To 10 cc. of neutralized filtrate and to 10 cc. of a solution containing a known amount of beryllium, add 1 drop of a 0.1 per cent alcoholic solution of curcumin, 0.5 cc. of 4 *N* ammonium chloride solution, and from 6 to 8 drops of 4 *N* ammonium hydroxide. Standard and unknown must be started simultaneously, as flocculation occurs on standing. Compare by the usual methods.

**Standard**—Prepare by dissolving a suitable salt in the same reagents

---

<sup>1</sup> I. M. Kolthoff, *J. Am. Chem. Soc.* 50, 393-5 (1928).

## BY QUINALIZARINE

used with the sample. Make additions to the standard corresponding to those added to the sample and to impurities known to be present in the sample. This is necessary as it is known that magnesium, fluorides and excess ammonium chloride interfere. Excess sodium hydroxide must be absent.

### BERYLLIUM BY AURIN TRICARBOXYLIC ACID

The presence of 0.02 mg. of beryllium per cc. gives a pink color but no precipitate with this reagent.<sup>2</sup> The colorimetric method of estimation has not been reported, although further commented on by Kolthoff.<sup>1</sup>

### BERYLLIUM BY QUINALIZARINE

Quinalizarine, 1, 2, 5, 8-hydroxyanthraquinone, gives a blue color with beryllium in alkaline solution. Unless an excess of alkali is present aluminum under the same conditions gives a violet lake. Excess of reagent is red-violet and therefore interferes.<sup>4</sup> A method has been developed in which the sample containing excess reagent is titrated with beryllium solution to a clear blue end point, indicating stoichiometric proportions of dye and beryllium.<sup>5</sup> It is questionable whether the colorimetric procedure is simpler.

Aluminum must be quantitatively separated<sup>6</sup> before determining beryllium. The precipitation of the color lake is incomplete in the presence of tartrates. Phosphates also interfere. The method may be used for as little as 0.1 mg. of beryllium oxide.

**Sample—Separation from Aluminum.** To separate aluminum from a solution containing aluminum and beryllium, acidify slightly with sulfuric acid. Dilute with hot water to 500 cc., or to 600–800 cc. if more than 0.1 gram of aluminum oxide is present. Warm a cold, saturated solution of ammonium acetate containing 3 grams of pure gallotannic acid per 100 cc. to about 80°. The solution must remain clear. Add a slight excess of this, with stirring, to the aluminum-beryllium solution. An aluminum hydroxide-tannin compound separates at once. Boil for 2 minutes, then let cool. Test the completeness of the precipitation in the

<sup>2</sup> A. R. Middleton, *J. Am. Chem. Soc.* 48, 2125 (1926).

<sup>4</sup> Hellmut Fischer, *Wiss. Veröff. Siemens-Konzern* 5, 99-119 (1926).

<sup>5</sup> Hellmut Fischer, *Z. anal. Chem.* 73, 54-64 (1928).

<sup>6</sup> L. Moser and M. Niessner, *Monatsh.* 48, 113-21 (1927).

supernatant liquid with a few drops of reagent. Filter and wash with a solution of ammonium acetate.

at the nitrate and washings containing the beryllium to boiling. Nitric acid in several small portions to oxidize the tannin. Continue until the solution becomes colorless. Neutralize with concentrated ammonia.

Transfer to a 100 cc. beaker  
 the hot solution  
 Wash with water  
 0.1 *N* sulfuric acid

more easily. Dissolve the lake in sodium hydroxide solution  
 or less of beryllium 25 cc. of 0.5 *N* hydroxide in water.

**Procedure**—To the solution of sample in sodium hydroxide add sufficient distilled water to bring the concentration to 0.25 *N*. Compare with a beryllium standard similarly prepared from beryllium oxide. The dye solutions should be examined as soon as possible. They change noticeably within 12 hours. The concentration of sodium hydroxide in sample and standard must be the same, as variation of alkalinity affects the color developed.

## CHAPTER XXXVII

### COLUMBIUM

#### COLUMBIUM BY REDUCTION OF THE FLUORIDE

THE only method available is one worked out for determination in tantalite. The blue fluoride compound of columbium is changed to brown when reduced with zinc in hydrochloric acid.<sup>1</sup> Tantalum does not interfere. The method is applicable to as low as 0.005 per cent. The glassware used will be etched by the hydrofluoric acid. Test tubes calibrated by the operator are therefore recommended for comparison.

**Sample**—Dissolve 10 grams of tantalite in 80 cc. of 1:3 hydrochloric acid with the addition of 15 cc. of 48 per cent hydrofluoric acid. Evaporate the clear solution to 40 cc. Large amounts of potassium fluotantalate will separate. Cool, filter, and wash the residue with 10 cc. of cold water containing 0.5 cc. of 48 per cent hydrofluoric acid. Potassium fluocolumbate remains in solution with a small amount of fluotantalate. Evaporate carefully to dryness on the sand bath. Dissolve in 10 cc. of concentrated hydrochloric acid. Rinse into a comparison tube with 5 cc. of water and dilute to 25 cc. with concentrated hydrochloric acid. If the sample contains more than 0.1 per cent of columbium the amount of sample should be correspondingly reduced.

**Procedure**—Reduce the sample solution with about 2 grams of zinc, added in several portions. For accurate work the standard cannot be diluted to match the sample. Several nonpermanent standards should be prepared at the same time as the sample and compared by the series of standards method. The standard is not stable but the time elapsing before it changes color has not been definitely determined.

**Standard**—Dissolve 5 grams of pure potassium fluotantalate and 0.005 gram or other suitable weight of columbium in 80 cc. of 1:3 hydrochloric acid and 15 cc. of 48 per cent hydrofluoric acid. Carry through the same procedure as the sample.

---

<sup>1</sup> E. Meimberg, *Z. angew. Chem.* 26, 83 (1913).

## CHAPTER XXXVIII

### GOLD

#### GOLD AS COLLOIDAL GOLD

A SMALL amount of gold salt in solution may be determined by reduction to the colloidal state. A colloidal solution of gold in the finest degree of dispersion is red. This is changed toward a blue modification of larger particle size known as "Purple of Cassius," by the presence of a small amount of electrolyte. The blue suspension finally becomes unstable and separates as a gelatinous mass on standing. The color produced on reduction of a gold salt is extremely variable depending on the conditions and the reducing agent used. The red gold sol has been obtained by Maxson<sup>1</sup> in the absence of added electrolytes, using acetylene as a reducing agent, and compared with that from varying amounts of pure gold chloride dissolved in hydrochloric acid. Formaldehyde, *m*-phenylenediamine, phenylhydrazine, benzidine and stannous chloride have also been used as reducing agents, the last most commonly. To estimate gold in cyanide solutions the cyanide must be removed before reduction. This is done by oxidation with sodium peroxide or with bromine, by combination with another ion, volatilization, or by precipitation in the presence of zinc dust and a lead salt. Methods may be used which are so delicate as to show the presence of one cent's worth of gold in a ton of water and by concentration of the solution being tested even greater delicacy may be secured. It is by these methods that the well known fact that every ton of sea water contains a few cents' worth of gold is proven.

**Sample**—For aqueous solutions free from interfering metals use 50 cc. Many types of solutions require purification as below. If too dilute, concentrate by evaporation.

*Cyanides.*<sup>2</sup> These must be treated under an efficient hood in all cases. To 50 cc. of solution add about 1.0 gram of potassium bromate. Add 1:1 sulfuric acid until effervescence ceases, usually about 2 cc. Boil off excess bromine, cool and dilute to 50 cc. By boiling for some time potassium

---

<sup>1</sup> R. N. Maxson, *Chem. News* 94, 257-8 (1906).

<sup>2</sup> H. R. Cassel, *Eng. Mining J.* 76, 661-2 (1903).

chlorate and hydrochloric acid can also be used for oxidation of cyanides. If the comparison is made quickly it is not necessary to boil off the bromine before addition of the reducing agent but the color will not remain at its true intensity for more than a minute unless the bromine is so removed.

*Cyanides. Alternative Treatment.<sup>2</sup>* To 50 cc. of sample add 1.0 gram of sodium or potassium bromide. When solution is complete add 1.0 gram of sodium peroxide. The liberated bromine decomposes the cyanide. Neutralize with sulfuric acid and add 1 cc. of concentrated hydrochloric acid.

*Cyanides. Alternative Treatment.<sup>3</sup>* Treat 50 cc. of solution with 15 cc. of concentrated ammonium hydroxide. Neutralize with concentrated sulfuric acid and add 1 cc. of concentrated hydrochloric acid.

*Cyanides. Alternative Treatment.<sup>5</sup>* To 100 cc. of solution add about 1.0 gram of sodium peroxide. Boil for 2 minutes to destroy cyanides. To insure excess peroxide add 2 drops of 10 per cent lead acetate solution. A brown precipitate of lead dioxide forms and redissolves if excess peroxide is present. If not add more peroxide. Remove the flame and add about 0.1 gram of aluminum powder. Stir until evolution of hydrogen ceases. A black precipitate of gold and lead is obtained. Filter through a small paper.

Warm a mixture of 4 cc. of concentrated nitric acid and 6 cc. of concentrated hydrochloric acid. Pour through the paper several times until the precipitate is dissolved. The color may be developed in concentrated solution or diluted to 50 cc. according to the strength of the sample. The smallest amount of lead possible should be used so that the precipitate to be redissolved will be small. Mercury if present will cause a dark color which will obscure the result.

*Cyanides. Alternative Treatment.<sup>6</sup>* Dissolve 10 grams of crystallized copper sulfate in 100 cc. of water. Add 20 grams of sodium chloride and 1 gram of copper shavings. Boil for 10 minutes, cool and add 5 cc. of 30 per cent acetic acid.

Acidify 100 cc. of solution under a hood with 1:1 hydrochloric acid and boil 1 minute to decompose cyanide. Add 5-10 drops of the copper solution. Test with paper moistened with potassium ferrocyanide. A red-brown color shows the presence of excess copper. To the boiling solution add 5 drops of a 2 per cent sodium sulfide solution. Boil this solution for 5 minutes, let settle, and decant through a filter. Dissolve the precipitate in the beaker in 25 cc. of a 5 per cent solution of sodium

<sup>5</sup> J. Moir, *Proc. Chem. Met. Mining Soc. of S. Africa* 4, 298-302 (1903).

<sup>6</sup> A. Prister, *Proc. Chem. Met. Mining Soc. of S. Africa* 4, 385 (1903).

cyanide to which has been added a few drops of 10 per cent sodium hydroxide solution. Pour through the filter and wash with a small volume of water. To the filtrate, which may be cloudy, add 2 grams of 200-mesh zinc dust and heat to 45° for one-half hour. The gold is precipitated. Decant through a filter. Dissolve excess zinc in 10 cc. of 1:1 hydrochloric acid and filter. Warm a mixture of 2.5 cc. of concentrated nitric acid and 7.5 cc. of concentrated hydrochloric acid. Pour through the filter paper 4 to 5 times to dissolve the gold. Wash with a small amount of water and dilute to 20 cc.

*Cyanides. Alternative Treatment.*<sup>7</sup> Place 500 cc. of the sample solution in a 1 liter bottle which has very little shoulder. Add 10–15 cc. of a saturated solution of sodium cyanide and 2–3 drops of a saturated solution of lead nitrate. Add 2 grams of 200-mesh zinc dust, stopper the bottle lightly and shake until the precipitate formed settles. This usually takes about 2 minutes. Invert into a casserole and decant the clear liquid. Add 1:1 hydrochloric acid to the precipitate drop by drop under a hood until the reaction ceases, and 0.5 cc. in excess. Evaporate to about 2 cc. Transfer to a small test tube.

*Ores Poor in Gold.* Moisten 100 grams of ore slightly but evenly in a glass-stoppered bottle with 1–2 cc. of equal volumes of bromine and ether. Shake at frequent intervals for 2 hours, during which time the interior of the bottle must be filled with bromine vapor. Add 50 cc. of water to the bottle and allow to stand for 2 hours, shaking occasionally. Filter and evaporate the filtrate to 20 per cent of its former volume. Add 5 cc. of saturated bromine-water and boil off excess bromine. Cool and dilute to 50 cc.

*Tissue.*<sup>8</sup> Digest with nitric and sulfuric acids, with or without addition of hydrogen peroxide according to the usual wet-ashing methods.<sup>9</sup> Evaporate excess acid, including sulfuric acid, to dryness. Dissolve the residue, which contains the gold as metal, in 0.5 cc. of concentrated hydrochloric acid and 1.5 cc. of nitric acid. Transfer to a volumetric flask and dilute to such a volume that 0.02–0.2 mg. of gold is present per cc. The sample was worked out for use with dimethylaminobenzylidenerhodanine as developing agent.

**Procedure—Stannous Chloride.**<sup>7</sup> This method for the determination of dilute gold solutions does not require the making up of a series of

<sup>7</sup> C. Brodigan, *Met. Chem. Eng.* 12, 460 (1914).

<sup>8</sup> B. K. Merejkovsky, *Bull. soc. chim. biol.* 15, 1336-8 (1933).

<sup>9</sup> See pp. 497-9.



standards but requires that the worker have sufficient knowledge of the colors after some use of the method to be able to estimate the gold content from the depth of color produced. A solution containing gold up to a value of 8 cents per ton may be used.

Dissolve 12.5 grams of crystallized stannous chloride in 30 cc. of 1:3 hydrochloric acid. Dilute to 100 cc. with distilled water. Keep 1-2 grams of tin in the bottle to insure its remaining in a reduced condition.

Concentrate the sample to about 2 cc. Add 1 cc. of stannous chloride solution to develop the color. If the quantity of gold is small this may take 2 to 3 minutes.

The colors produced from a 100 cc. sample are as follows:

- 2 cents per ton—very slight color.
- 3 cents per ton—slight yellow color.
- 4 cents per ton—slight pinkish yellow color.
- 6 cents per ton—strong pink color.
- 8 cents per ton—purple of Cassius.

*Stannous Chloride. Alternative Procedure.* To the sample solution add one-fourth its volume of stannous chloride solution as prepared for the preceding method and mix. As in the preceding case, experience with solutions of known gold content is necessary in order for the operator to become familiar with the colors produced. Neither dilution nor balancing methods are reliable so the estimation must be based on observation of standards, and experience. The gold standard used should be diluted to the volume of the sample with a solution containing about 3 parts by volume of concentrated hydrochloric acid and 1 part by volume of concentrated nitric acid to 6 parts of water.

For ore solutions the following colors have been reported by this method.

- 0.1 per cent solution, deep brown color, opaque even in thin layers.
- 0.01 per cent solution, brown violet immediately, 14 cm. column opaque.
- 0.001 per cent solution, pale violet immediately, increases after a time.
- 0.0001 per cent solution, evaporated to one-fifth former volume with a drop of bromine water added, a distinct rose tint in a 14 cm. column.
- 0.00005 per cent solution, faint but recognizable pink in 14 cm. column, after undergoing the same treatment.

*Metaphenylene Diamine.*<sup>11</sup> To a 50 cc. sample add 5 cc. of 0.5 per cent

<sup>11</sup> J. A. Siemssen, *Chem.-Ztg.* 36, 934

solution of metaphenylene diamine. Add 5 cc. of 1:1 sulfuric acid. A yellow to dark brown color is formed, dependent on the strength of the gold solution. A solution as dilute as 0.005 per cent will show a color by this method. If the reagent becomes pink on standing in the light decolorize with activated carbon.

*Formaldehyde.*<sup>12</sup> To 50 cc. of sample add sufficient 20 per cent sodium hydroxide solution for neutralization, and 1 cc. in excess. On addition of 1 cc. of 40 per cent formaldehyde a blue color is developed. The investigators of the method were able to use the balancing method but recommended colored glass standards. The optimum concentration for this determination is 1 part in 40,000.

*Phenylhydrazine.*<sup>13</sup> To the sample add 1 gram of citric acid and 5 drops of a 10 per cent solution of phenylhydrazine hydrochloride. A blue-violet by transmitted light is obtained. This will detect one part in 2 million. Comparison with a series of standards is advisable. Such standards keep several hours, but eventually give a black deposit.

*Benzidine.*<sup>14</sup> As reagent dissolve 1 gram of benzidine in 50 cc. of 20 per cent acetic acid. Add 1 cc. of this to the sample and compare with similar standards. Iron and platinum must be absent. The reagent detects 2 parts of gold per million.

*Dimethylaminobenzylidenerhodanine.*<sup>15</sup> To prepare the reagent add 0.01 gram of dimethylaminobenzylidenerhodanine and 0.5 cc. of 33 per cent potassium hydroxide solution to 10 cc. of absolute alcohol. Dissolve as much as possible by shaking, and filter. The reagent is ready for use and keeps only 24 to 48 hours.

Transfer 2, 1, 0.5, 0.3 and 0.1 cc. of sample solution to tubes and dilute each to 2 cc. Add 2 drops of a 0.5 per cent solution of agar-agar and 1 drop of 33 per cent potassium hydroxide solution to each. Mix and add 4 drops of reagent. Mix and add 1 drop of concentrated hydrochloric acid. A lake forms at once and is stable. Compare with a standard or series of standards similarly prepared. The method will detect 0.005 mg. in 2 cc. Iron or dilute acid does not interfere. Some heavy metals do interfere.

*Acetylene.*<sup>1</sup> Prepare a saturated aqueous solution of acetylene. To a 50 cc. sample add 10 cc. of this reagent. If electrolytes are absent a red color is obtained. In their presence the color is blue and varies with the

<sup>12</sup> A. Muller and A. Foix, *Bull. soc. chim.* 31, 717-20 (1922).

<sup>13</sup> M. Pozzi-Escot, *Ann. chim. anal.* 12, 90-1 (1907).

<sup>14</sup> G. Malatesta and E. di Nola, *Boll. chim.-farm.* 52, 461 (1912).

<sup>15</sup> B. K. Merejkovsky, *Bull. soc. chim. biol.* 15, 1336-8 (1933).

concentration of electrolyte. This reducing agent is probably inferior to the others previously given.

**Standard**—Dissolve 3.11 mg. of pure gold in 10 cc. of aqua regia and dilute to 1 liter. This corresponds to 48 grains of gold per ton of solution. Keep in a dark bottle. If results are to be reported in p.p.m. use 5 mg. of gold and the final solution contains 5 p.p.m.

#### GOLD BY MERCUROUS CHLORIDE

In the absence of interfering metals, the effect of gold in coloring mercurous chloride may be used for its colorimetric estimation.<sup>16</sup> The color is due to the reducing action of mercurous chloride. Arsenic, platinum, palladium, selenium, tellurium and iodine must be absent in the final sample. Colored solutions do not interfere and the reaction is stated to be more sensitive than the others in use. Since it depends on the colloidal color, it is hardly apt to be more sensitive, but it should be applicable in places where the other methods are not. In general, the interfering ions listed above are not often present in substantial amounts in gold solutions. Over 0.003 gram of copper or 0.006 gram of iron will interfere.

**Sample**—For separation from interfering elements see the application of the same method to platinum.<sup>17</sup>

**Procedure**—Add about 0.1 gram of mercurous chloride to 5 cc. of distilled water. Mix and add sufficient neutral or slightly acid sample to give a definite color, and mix. Stir occasionally until the reaction is complete and let the precipitate settle. Compare with standards similarly prepared. The colors produced are the following:

Gold in mg.	Color on Mercurous Chloride
0.20	Dark purple
0.10	Pinkish purple
0.05	Purplish pink
0.02	Strong pink
0.002	Light pink
0.0002	Very light pink
0.00005	Faint coloration

<sup>16</sup> Gordon G. Pierson, *Ind. Eng. Chem., Anal. Ed.* 6, 437-9 (1934).

<sup>17</sup> See pp. 420-1.

GOLD BY *o*-TOLIDINE

This well known reagent for free chlorine is also a sensitive test for chlorauric acid.<sup>18</sup> One p.p.m. of gold gives a bright yellow and 0.1 p.p.m. gives a yellow color detectable in a depth of 10 cm. Ferric iron, ruthenium, osmic acid, vanadates, tungstates, nitrous acid and free chlorine must be absent. If copper is present and gives a green color, tint the standard with copper to match before adding the reagent to either.

**Procedure**—Render 25 cc. of the solution slightly acid with hydrochloric acid. Add 1 cc. of a solution of 0.1 g. of *o*-tolidine in 100 cc. of 1:10 hydrochloric acid. Mix well and compare after 3 minutes with a standard similarly prepared at the same time. The color fades slowly after one-half hour.

---

<sup>18</sup> W. B. Pollard, *Analyst* 44, 94-5 (1919).

## CHAPTER XXXIX

### SILVER

#### SILVER AS THE CHLORIDE

THE comparison of silver as the chloride has been extensively used for the nephelometric estimation of small amounts, particularly in atomic weight work.<sup>1,2</sup> The procedure is affected by the presence of foreign electrolytes.<sup>3</sup> It has been reported as unsatisfactory for nephelometric use,<sup>4</sup> a statement which must be given only limited weight in view of the numerous investigators who have used the method with apparent success. Weight must be given to the statement<sup>4a</sup> that, even with a special type of photronic colorimeter, for highest accuracy it is easier to use electrometric methods. The limiting factors as to accuracy have been reported as instrumental<sup>5</sup> and as the reproducibility of the suspension,<sup>6,3</sup> Standards must be freshly prepared.<sup>1</sup>

There is a tendency for shaking and cooling to leave an excess of chloride ion in solution, which may amount to several tenths of a mg. per liter.<sup>9</sup> The sources of the errors are suggested as adsorption of ions by silver chloride during peptization and coagulation, adsorption on the precipitate, differences in the coagulating action of the two precipitating reagents and peptizing and coagulating effects of other compounds present.

The rate of mixing is highly important. Comparison of pouring, stirring and "instantaneous" mixing shows that the more rapidly the mixing takes place the less the turbidity. However, the reproducibility is better with the slower methods of mixing, which is more important.<sup>10</sup> Further

---

<sup>1</sup> T. W. Richards and R. C. Wells, *Am. Chem. J.* 31, 235 (1904).

<sup>2</sup> A. B. Lamb, P. W. Carleton and W. B. Meldrum, *J. Am. Chem. Soc.* 42, 253 (1920).

<sup>3</sup> I. M. Kolthoff and Henry Yutzy, *J. Am. Chem. Soc.* 55, 1915-22 (1933).

<sup>4</sup> Hans Kleinmann, *Biochem. Z.* 99, 115-49 (1919).

<sup>4a</sup> H. Howell Furman and George W. Low, Jr., *J. Am. Chem. Soc.* 57, 1588-91 (1935).

<sup>5</sup> P. A. Kober, *J. Biol. Chem.* 13, 490 (1915).

<sup>6</sup> P. V. Wells, *Chem. Rev.* 3, 331-82 (1927).

<sup>9</sup> Clyde R. Johnson, *J. Phys. Chem.* 35, 540-2, 830-5, 2237-44, 2581-4 (1931).

<sup>10</sup> Arthur F. Scott and John L. Moilliet, *J. Am. Chem. Soc.* 54, 205-9 (1932).

development of special equipment permits reproducibility with very rapid mixing.<sup>11</sup> A photronic nephelometer has been constructed for study of the problem.<sup>12</sup>

Although a strictly nephelometric procedure, the method is included briefly here because of its importance.

**Procedure**—To 20 cc. of acid or neutral solution containing silver ion add 10 cc. of 0.1 *N* nitric acid. Then add 10 cc. of 0.005 *N* hydrochloric acid. Mix well, heat on a water bath at 40° for 30 minutes, cool rapidly to room temperature and compare nephelometrically with a standard prepared at the same time in the same way. A suitable standard is 5 cc. of silver nitrate solution containing 0.1 p.p.m. of silver and 15 cc. of water. The final solution will contain 0.0125 p.p.m. of silver.

**Standard**—Dry pure silver nitrate crystals in a desiccator. Dissolve 0.1575 gram in water and dilute to 1 liter. Each cc. corresponds to 0.1 mg. of silver. Dilute 100 cc. of this to 1 liter to give a solution containing 10 p.p.m. Repeat this operation to get solutions containing 1 p.p.m. and 0.1 p.p.m.

#### SILVER AS THE SULFIDE

Silver has been estimated in lymph nephelometrically as the sulfide.<sup>13,14</sup> The method is analogous to the estimation of lead as the sulfide and appears to be adaptable to other samples. Iron and lead do not interfere.

**Sample**—Dry a sample of lymph on the water bath. Ash carefully and fuse with a small amount of sodium nitrate until the residue is white. Heat twice for 5 or 10 minutes with 5 cc. portions of 1:1 nitric acid to dissolve everything but silver chloride. Pour the liquid into a comparison tube. Wash the residue twice with 5 cc. of 10 per cent ammonium hydroxide, and put into the comparison tube.

**Procedure**—Make the sample solution faintly ammoniacal, dilute to about 70 cc. with water, and add 10 cc. of clear hydrogen sulfide solution. Acidify with nitric acid to dissolve the iron sulfide from any blood present

<sup>11</sup> Arthur F. Scott and Frank H. Hurley, *J. Am. Chem. Soc.* 56, 333-5 (1934).

<sup>12</sup> Charles H. Greene, *J. Am. Chem. Soc.* 56, 1269-72 (1934).

<sup>13</sup> P. W. Danckwortt, *Arch. Pharm.* 252, 29-76 (1914).

<sup>14</sup> Tibor v. Heidelberg, *Biochem. Z.* 192, 233-40 (1928).

and dilute to 100 cc. Into a second tube put a suitable amount of a standard silver nitrate solution. Add 10 cc. of 10 per cent ammonium hydroxide and dilute to about 70 cc. Add 10 cc. of hydrogen sulfide solution, acidify with nitric acid and dilute to 100 cc. Compare by dilution or by balancing.

#### SILVER BY REDUCTION WITH HYPOSULFITE

When a dilute ammoniacal silver solution containing gelatine is treated with sodium hyposulfite,<sup>15</sup> a clear yellow sol forms. It is suitable for colorimetric estimation of silver.<sup>16</sup> The method is applicable to 1–20 mg. per liter. Copper, cobalt, nickel and cadmium interfere, and must first be removed. The distilled water used must give a negative diethyl-dithiocarbamate test.

**Sample**—*Cobalt, nickel, cadmium or copper present.* To a known volume of a solution containing silver add an excess of bromine-water and hydrobromic acid. Evaporate until the silver bromide coagulates. Filter the precipitate, which may also contain lead or thallium, on an inorganic filter. Wash with water.

Place the crucible in a beaker and add 10 cc. of concentrated ammonium hydroxide. Cover with a watch glass and let stand for 4 hours. Wash the crucible with 1:100 ammonium hydroxide and dilute the resulting solution and washings to a silver concentration of about 10 mg. per liter.

*Cobalt, nickel, copper and cadmium absent.* Transfer 200 cc. of sample solution to a 250 cc. volumetric flask. Add 5 cc. of bromine-water, mix and set aside for 3 minutes. Add 2 cc. of 10 per cent diammonium phosphate solution and 0.5 cc. of saturated sodium hyposulfite solution. Mix, add 2 cc. of concentrated ammonium hydroxide and mix again. Dilute to 250 cc. and after 10 minutes filter through a dry filter. Reject the first 25 cc. of filtrate. This treatment removes magnesium, calcium, iron and aluminum.

**Procedure**—Prepare an ammoniacal gelatine solution as follows: Soak 2 grams of ash-free gelatine in 100 cc. of water for a few hours. Then heat to dissolve. Add 100 cc. of concentrated ammonium hydroxide, di-

<sup>15</sup> The term hyposulfite refers to the salts of  $\text{H}_2\text{S}_2\text{O}_4$  and is not to be confused with the older term of hyposulfite for salts of  $\text{H}_2\text{S}_2\text{O}_3$ , commonly known as thiosulfates.

<sup>16</sup> E. E. Jelley, *J. Soc. Chem. Ind.* 51, 191-3T (1932).

lute to 1 liter and hold at 95° for 6 to 7 hours. If ash-free gelatine is not available, add 0.2 gram of diammonium phosphate to precipitate calcium and magnesium.

Transfer 10–40 cc. of the ammoniacal filtrate of sample to a 50 cc. volumetric flask. Add 10 cc. of the gelatine solution and 0.04 gram of dry sodium hyposulfite. Dilute to 50 cc. with *N* ammonium hydroxide. Shake and pour into a dry tube. Warm to 50° in a water bath to develop the color. Compare with a standard similarly treated.

**Standard**—To 9.25 cc. of 0.1 *N* silver nitrate solution add 25 cc. of concentrated ammonium hydroxide and dilute to 1 liter. Each cc. contains 0.1 mg. of silver.



## CHAPTER XL

### PLATINUM

#### PLATINUM BY REDUCTION TO PLATINOUS CHLORIDE

PLATINUM in solution may be determined by reducing a solution of platinic chloride to the dark red to brownish red platinous chloride. The color of platinous chloride is inversely proportional to the acidity. It is therefore very important that the acidity of sample and standard be the same. If the acidity is less than 0.1 *N* precipitation occurs.

The greatest probable error is 10 per cent. For more than 0.2 mg. of platinum micro gravimetric methods are more accurate.

**Sample—Ores,<sup>1</sup> Sands or Concentrates.** Grind the sample to pass a 100 mesh screen, or if difficultly fusible substances such as chromite or zircon are present, grind to pass a 150 mesh screen. Mix one assay ton of the carefully sampled ore with a suitable flux. Table 4 of a number of charges gives typical examples. All charges are in grams.

TABLE 4. CHARGES FOR SEPARATION OF PLATINUM BY FIRE ASSAYING.

		Hem.	Lim.	R. red	Black sand, magnetic iron	Chrom.		Sulfa Barite and
Ore .....	29.166	29.166	29.166	29.166	29.166	29.166	29.166	29.166
Sodium Carbonate. 30-60	30	.....	40	.....	.....	.....	30-60	.....
Sodium Bicarbonate ....	.....	.....	40	.....	20	60	.....	30
Borax .....	.....	.....	20	20	.....	.....	10	20
Borax Glass .....	5	8	.....	.....	30	20	.....	.....
Litharge .....	60-90	70	40	40	66	35	50-150	150
Silica .....	.....	7	5	4	.....	15	0-10	.....
Fluorspar .....	.....	.....	.....	.....	.....	.....	.....	15
Argols .....	2.5	3.5	2.5	7	4	3	.....	.....
Niter .....	.....	.....	.....	.....	.....	.....	21	8
Sodium Chloride ..	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

<sup>1</sup> C. W. Davis, *Bur. Mines Tech. Paper 270*, 12 (1921).

The quantity of niter or argols is only approximate in each case and should be so varied as to give a 30 gram lead button. A borax glass cover layer is used for each.

If 15 times as much silver as platinum is not present add silver chloride or nitrate to make up the deficiency. Excess silver is required to render the platinum soluble in nitric acid when parting, to assist in removing the last traces of lead in cupelling, and to diminish loss in the cupel. For an unknown ore use 0.05 gram of silver chloride.

Flux as in the ordinary fire assay for gold and silver but after the fusion is quiet raise the temperature higher than usual and continue heating for an hour. Remove the crucible and let cool without agitating. Break the button free from slag and cupel at a high temperature. This causes loss of silver so that it must be separately determined.

When the platinum constitutes more than 1.6 per cent of the bead, the latter has a frosted appearance. Iridium causes a roughness of finer texture. Palladium gives the bead an embossed appearance. Ruthenium if present in quantity, turns the surface a bluish black, leaving a black scum on the cupel. After cupelling, part the button with nitric acid, first with 1:4 acid, then 1:1, and finally 2:1. If gold, silver, and all the platinum metals are in the ore, silver, palladium, and platinum are dissolved by this treatment, leaving gold, iridium, rhodium, and some ruthenium and osmium. Most of the osmium and part of the ruthenium are oxidized and lost during cupellation. Part of the iridium may not collect in the silver and will be lost on the cupel. If considerable platinum is found some will remain undissolved. Filter the residue on an ashless paper, ignite and save for the recovery of platinum as well as for the determination of iridium and rhodium.

Add dilute hydrochloric acid to the filtrate slowly with constant stirring, to precipitate silver. Let stand over night, filter off the silver chloride and wash with 1:20 nitric acid. If the precipitate is pink redissolve in the minimum amount of 1:1 ammonium hydroxide and reprecipitate with 1:1 hydrochloric acid to recover occluded platinum or palladium.

Evaporate the filtrate just to dryness. Take up with 10 cc. of 1:5 hydrochloric acid and again evaporate just to dryness. Take up with 10 cc. of 1:5 hydrochloric acid, transfer to a 30 cc. beaker, and evaporate nearly to dryness. When cold take up with 5 cc. of cold water and filter on a 5 cm. paper to remove the last trace of silver. Make the filtrate slightly alkaline with sodium carbonate, add 1 cc. of 30 per cent formic acid and boil in a covered beaker until all the platinum and palladium are

precipitated. This will usually require about 30 minutes. Filter on a 5 cm. paper, wash with hot water and ignite in an "impervite" crucible. The platinum metals adhere to both glazed and unglazed porcelain. Add 1 cc. of formic acid to the filtrate and boil again to insure complete precipitation. If the first parting solution is yellow or orange, the presence of palladium is indicated. Warm the metals with 1:4 nitric acid which dissolves the palladium, filter, wash, and ignite the platinum residue. For treatment to recover palladium see method for that element.

Treat the residue from the nitric acid parting, which may contain gold, iridium, rhodium, and some ruthenium, osmium, and undissolved platinum, with 5 cc. of 1:5 *aqua regia*, thus dissolving the gold and platinum. Filter on a 5 cm. paper. Evaporate the acid solution of gold and platinum to dryness. Take up with 5 cc. of 1:1 hydrochloric acid, and again evaporate to dryness. Treat a second time with 5 cc. of 1:1 hydrochloric acid and evaporate nearly to dryness. Take up with 5 cc. of cold water and filter if necessary. Add 0.1 gram of oxalic acid and boil until the gold is all precipitated. It is best to let the solution stand over night before filtering off the gold. After filtering, neutralize the filtrate with 20 per cent sodium carbonate solution, boil with 1 cc. of 30 per cent formic acid, filter off the platinum and ignite. Combine with the platinum previously separated.

If the total platinum amounts to more than 0.2 mg. it may be determined by weight. If less than 0.2 mg. dissolve the ignited platinum in 5 cc. of *aqua regia* and evaporate just to dryness, twice, with hydrochloric acid. Extract with a stock solution of hydrochloric acid containing 33 cc. of concentrated acid diluted to 1 liter, using 2 cc. of this acid for every 0.2 mg. or less of platinum.

If the quantity of platinum is small, run several samples of an assay ton each, combine the lead buttons, scorify to about 30 grams and proceed as above. As a check on the raw materials add the approximate amounts of gold, silver and platinum to the crucible charge and carry through the same procedure.

*Filings and Sweeps.* Dissolve a suitable sample according to concentration in 10 cc. of *aqua regia*. Evaporate to dryness and take up with 10 cc. of 1:5 hydrochloric acid. Add about 1 gram of 20 mesh zinc and warm if necessary to hasten the action.

Filter off the precipitated metals, wash and dissolve all except gold and platinum with cold 1:5 nitric acid. Filter off the gold and platinum. The removal of copper, always found in such materials as jewelers' filings, is important as it affects the final color. Wash the deposit of gold and

platinum thoroughly to remove all the copper nitrate. Dissolve the gold and platinum in 10 cc. of *aqua regia* and evaporate to dryness. Take up with 10 cc. of 1:20 hydrochloric acid. Prepare a ferrous sulfate solution by dissolving 2 grams in 100 cc. of 1:20 hydrochloric acid. Add 2 cc. of this solution, free from ferric ion, to the platinum and gold solution. Ferric ion will cause a yellow color. Warm to 40°, but not higher, and maintain at that temperature for 45 minutes. As an alternative it may be allowed to stand for 3 to 4 hours but heating, which coagulates the precipitate, is preferable. Not over 0.2 gram of ferrous sulfate should be present per mg. of gold. Gold is precipitated and carries with it a trace of platinum. The amount so carried down is very small.

Filter and evaporate the filtrate just to dryness. Take up with the stock hydrochloric acid as in the previous method. An alternative method of separation of gold is with oxalic acid as given for ore samples.

*Mixed Platinum Metals.*<sup>1a</sup> By this procedure the elements are separated in the following order: Osmium, ruthenium, platinum, palladium, rhodium, iridium. Details are given here for the complete separation, some of which apply to later chapters. While the separation is designed for gravimetric use it is believed to be applicable to smaller amounts which will then necessarily be estimated colorimetrically. Accuracy within 0.1 mg. has been obtained on 100–300 mg. quantities and can be improved by micro manipulation. Because of the variability in size of sample, details of the exact amounts of reagents are not given.

*Separation of Osmium.* Acidify the sample so that it contains 10 per cent of nitric acid by volume. Transfer to a 700 cc. distilling flask having inlet and delivery tubes sealed in and connected to a set of three 300 cc. absorbing flasks by ground glass joints. Depending on the size of sample, the size of this equipment may be proportionately reduced. Fill the absorbing bottles with 6 *N* hydrochloric acid saturated with sulfur dioxide. Heat the sample solution to boiling and pass a gentle current of air through it until the osmium tetroxide is completely carried over. Combine the absorbing solutions and evaporate to a syrup to recover the osmium in the sample for further treatment.

*Separation of Ruthenium.* Evaporate the osmium-free residue from the flask on a steam bath to eliminate nitric acid. Add a few cc. of concentrated hydrochloric acid and evaporate to remove oxides of nitrogen. Repeat several times. Add 10 cc. of concentrated sulfuric acid and heat until sulfur trioxide fumes are evolved. Return the solution and any

---

<sup>1a</sup> Raleigh Gilchrist, *Technical News Bulletin of the National Bureau of Standards*, June, 1935, 62-3.

metallic platinum to the distilling flask and dilute to about 100 cc. Add sodium bromate in excess and distil as before absorbing the distilled ruthenium tetroxide in 6 *N* hydrochloric acid saturated with sulfur dioxide. To recover the ruthenium, concentrate the absorbing solution as described for osmium.

*Separation of platinum.* Transfer the solution from the distilling flask and neutralize to pH 6.0 with 1:1 ammonium hydroxide.<sup>1b</sup> Add sodium bromate to the solution if not already present and heat to boiling to precipitate the hydrated oxides of palladium, iridium and rhodium. Filter and redissolve the precipitate in 1:1 hydrochloric acid and reprecipitate the hydrated oxides to recover adsorbed platinum. Save the oxides for further treatment and combine the platinum filtrates. Acidify and add hydrogen peroxide to destroy excess bromate. Boil to decompose excess hydrogen peroxide.

As standard add to water similar materials to those added to the sample, which will vary with the processing up to this point. Add the standard solution of platinum to this and dilute to the same volume as the sample.

*Separation of palladium.* Dissolve the mixed hydrated oxides in 1:1 hydrochloric acid and dilute with water to a reasonable acidity. Add a solution of dimethylglyoxime in excess to precipitate the palladium. Filter and ignite the precipitate to decompose the glyoxime. Dissolve the metal or oxide in *aqua regia* and evaporate just to dryness. Take up the residue in 1:1 nitric acid and use as for nitric acid-parting solutions.

*Separation of rhodium.* Add 10 cc. of concentrated sulfuric acid to the filtrate from palladium glyoxime. Evaporate to a small volume and destroy excess of dimethylglyoxime by small volumes of concentrated nitric acid. Heat to fumes of sulfur trioxide to drive off nitric acid. Dilute to a suitable volume, such as 200 cc. and heat to boiling. Add a slight excess of titanous chloride<sup>1c</sup> to precipitate metallic rhodium. Filter and dissolve the precipitated metal in a small volume of boiling 1:1 sulfuric acid. Dilute to a suitable volume and again precipitate rhodium. Filter and dissolve the residue in the minimum possible volume of hot 1:1 sulfuric acid. Use this solution as sample.

*Separation of iridium.* Adjust the pH of the combined filtrates containing iridium to pH 6. Add an excess of cupferron solution to precipitate titanium. Filter and boil to precipitate the hydrated oxide of

<sup>1b</sup> Fr suitable indicators for various pH values see pp. 677-86.

<sup>1c</sup> See p. 378.

iridium. If interference by the excess of cupferron occurs evaporate to dryness, destroy the organic matter by heating with nitric acid and again take up to a suitable volume, adjusting to pH 6.

Dissolve the precipitate of hydrated iridium oxide in *aqua regia* and evaporate to dryness. Add a few drops of hydrochloric acid and again evaporate just to dryness. Take up with distilled water and use as sample.

*Platinum Solutions.* Solutions from plating or other sources should be treated as given for the solutions of filings or sweeps.

**Procedure**—Dissolve 5 grams of crystallized stannous chloride in 10 cc. of the stock hydrochloric acid solution, 33 cc. of concentrated acid per

sample and standards add 2 drops of this freshly prepared stannous chloride solution. Compare after 15 minutes by dilution with the stock hydrochloric acid solution or by balancing. After allowing that time for development, the color varied with the stannous chloride concentration was due to variation of acidity.

**Standard**<sup>2</sup>—Clean a soft sheet of platinum with concentrated nitric acid, then with concentrated hydrochloric acid, and finally heat to a bright red. Dissolve exactly 0.1000 gram of clean platinum in *aqua regia*, to 10 cc. of concentrated hydrochloric acid and evaporate just to dryness and distilled should be

0.1 gram  
m. By ( )  
is obtained which contains 0.1 plat-

<sup>2</sup> Lothar Wöhler, *Chem.-Ztg.* 31, 938 (1907).

<sup>3</sup> E. G. R. Ardagh, F. S. Seaborne, and N. S. Grant, *Can. Chem. Met.* 8, 117-20, 140-2 (1924).

inum per cc. The latter tends toward deposition after some weeks. This may be prevented by adding 5 cc. of concentrated hydrochloric acid.

If a high degree of accuracy is required neutralize 100 cc. of the dilute standard platinum solution with sodium carbonate and add 5 cc. of 30 per cent formic acid. Boil to precipitate the platinum, filter and ignite for gravimetric determination.

Evaporate suitable amounts of this dilute standard platinum solution just to dryness and take up with stock hydrochloric acid, as with the sample, using 2 cc. for every 0.2 mg. of platinum. The color is to be developed in these at the same time and in the same way as the sample.

#### PLATINUM AS THE IODIDE

Platinum may be determined by changing the platinic chloride to platinic iodide which dissolves in excess potassium iodide solution to give a rose to red color.<sup>2,5</sup> Sulfites, thiosulfates, ammonium hydroxide, and mercuric chloride destroy the color of the iodide.<sup>3</sup> The method will detect 1 part of platinum in 2 million parts of solution. The highest accuracy is obtained when 0.2 mg. of platinum is present in a 50 cc. sample.

**Procedure**—Prepare the sample as for the preceding method.<sup>7</sup> After taking up with the stock hydrochloric acid dilute to nearly 50 cc. with distilled water. Add 1 cc. of a 2 per cent solution of potassium iodide to standard and sample and complete the dilution to 50 cc. The color may be compared after an hour, although it is then only about 90 per cent of the maximum. The rate of development after an hour's standing is very slow. The color does not develop as readily with fresh solutions as with those which are two weeks or more old. The maximum is the same.

**Sources of Error**—Heating the solutions causes the color to develop more rapidly, but is to be avoided. At 50° the same maximum is not reached. Sulfuric acid hastens the development of color but fading occurs more quickly. Nitric acid causes a yellow to green color. Acetic acid retards or prevents appearance of the color. The optimum amount of

<sup>5</sup> J. C. H. Mingaye, *Records Geol. Surv. N. S. Wales* 8, 276 (1909).

<sup>7</sup> See pp. 413-18.

hydrochloric acid for 0.05–0.2 mg. of platinum hydrochloric acid. In the absence of acid the in 16 hours. Fading, when it occurs, is not due to

made

ate and 1 cc. of concer

. This is equivalent to 5.9 grams of the anhydrous salt o  
s of the heptahydrate. As dichromate standard, dissolve 0.1 gr  
sium dichromate and 1 cc. of concentrated sulfuric acid in water and  
dilute to 100 cc. Add the dichromate standard to the cobalt standard un-  
til the desired tint is obtained. The 1–4 p.p.m. solutions are particularly  
easily matched.

For 50 cc. samples and standards the following mixtures are suitable.

Platinum in mg per 50 cc.	Cobalt Standard in cc. per 50 cc.	Bichromate Standard in cc. per 50 cc.
0.1	3.5	1.5
0.2	9.0	3.0
0.3	14.5	4.5

Color matching in the Lovibond tintometer is also satisfactory. Better results have been reported with a special 4 inch tintometer cell than with platinum standards. A series of readings plotted is most satisfactory. Readings at full color are desirable. The results on a solution containing 0.2 mg. per 50 cc. sample are as follows for the 4 inch cell.

Time	Red	Yellow
1 minute	7.40	5.00
5 minutes	10.75	6.50
10 minutes	12.10	6.75
60 minutes	13.15	7.30
3 hours	13.55	7.70
16 hours	15.10	8.80



## PLATINUM AND PALLADIUM BY MERCUROUS CHLORIDE

The reducing action of mercurous chloride on platinum and palladium solutions can be used for coloration of the residual mercurous chloride, and their estimation.<sup>8a</sup> Arsenic, gold, selenium, tellurium and iodine give the same reaction. Interference of palladium and platinum can be avoided by a suitable procedure. More than 0.003 gram of copper interferes with platinum in the cold but this interference is eliminated by boiling. More than 0.003 gram of iron interferes with either platinum or palladium. For convenience the separation of gold from the sample solutions is also given below.

**Sample**—*Separation of gold, platinum and palladium.* Add sufficient oxalic acid to make the solution a 5 per cent solution of that acid and boil. Gold is precipitated. Filter on fine paper and wash. Dissolve the precipitate from the paper and beaker with 1:10 hydrochloric acid containing free chlorine. Boil to drive off free chlorine and use as sample.

Acidify the filtrate containing platinum and palladium with a few drops of concentrated sulfuric acid and evaporate nearly to dryness to decompose oxalic acid. If the residue is not completely soluble in water acidify with 1:10 hydrochloric acid containing free chlorine. In that case boil to dissolve, evaporate to dryness on a water bath and take up with water. The final acidity must not exceed 4 per cent as hydrochloric acid. Add 5 per cent by weight of crystallized copper sulfate and use for estimation of palladium and platinum.

**Procedure**—Transfer 0.1 gram of mercurous chloride and 5 cc. of water to a 100 cc. beaker. Mix and add sufficient neutral or acid solution of platinum, free from interfering elements, to give a definite coloration. Mix well and if a color develops it may be platinum or palladium. If palladium is thought to be present repeat in the presence of 0.003 gram of copper as the sulfate. If the color does not develop in the cold under these conditions it will develop on heating to boiling and is due to platinum. By suitable manipulation either platinum or palladium can be estimated in the presence of the other by comparison with suitable standards.

<sup>8a</sup> Gordon G. Pierson, *Ind. Eng. Chem., Anal. Ed.* 6, 437-9 (1934).

The colors developed by platinum are as follows :

Platinum, mg.	Color on Mercurous Chloride
0.1	Dark grey
0.02	Grey
0.01	Cream grey
0.005	Greyish cream
0.001	Cream
0.0002	Slight cream

Those developed by palladium are as follows :

Palladium, mg.	Color on Mercurous Chloride
0.2	Very dark grey
0.05	Grey
0.01	Light grey
0.002	Cream grey
0.0004	Greyish cream
0.00005	Faint cream

## CHAPTER XLI

### RHODIUM

#### RHODIUM BY STANNOUS CHLORIDE

THE concentration of comparatively pure solutions of rhodium may be estimated by means of the rose-red color produced with stannous chloride.<sup>1,2</sup> The first effect of adding stannous chloride to the boiling solution of the metal is production of a brown colloidal solution of the metal, analagous to the reactions of gold and platinum in the cold. The crimson color is probably due to the colloidal particles gradually going into solution in an acid medium. The greater the amount of rhodium present the longer the time necessary for full development of the color.

From 0.0005–0.001 mg. per cc. may be detected. The procedure is only in a generalized form and doubtless will require elaboration to obtain a high degree of accuracy.

**Sample—Solids.** Fuse a sample of suitable size with potassium acid sulfate. Extract the melt with water, filter, and evaporate the filtrate to 1 cc.

**Solutions.** Evaporate a suitable amount of the solution to 1 cc.

**Mixed Platinum Metals.** The method of separation of rhodium from osmium, ruthenium, platinum, palladium and iridium is given under platinum.<sup>2a</sup>

**Procedure—**To the acid solution of the rhodium salt add 1 cc. of a 40 per cent solution of stannous chloride in 30 per cent hydrochloric acid. Heat to boiling and cool. A brown color is developed on heating, but this changes to red as the solution cools. The color change may take an hour but once produced, the red color is quite stable. Compare with a suitable series of standards similarly treated. Add similar amounts of acid sulfate and other substances present in the sample to the standards in order that the quality of color may not vary.

---

<sup>1</sup> V. N. Ivanov, *J. Russ. Phys. Chem. Soc.* 49, 601-3 (1910).

<sup>2</sup> W. Singleton, *Ind. Chemist* 3, 121 (1927).

<sup>2a</sup> See p. 417.

## RHODIUM IN HYDROCHLORIC ACID

may be estimated by the

cobalt a sky blue, copper, iron and osmium golden ye  
and iridium give brown. Purification is therefore com  
and full details are not available for a concise method.

---

<sup>3</sup> F. Mylius and A. Mazzucchelli, *Chem. News* 112, 90-1 (1915).

## CHAPTER XLII

### IRIDIUM

#### IRIDIUM BY BENZIDINE

IRIDIUM gives a blue color with benzidine and acetic acid, similar to that given by platinum but deeper. Khlopin uses this to determine iridium in platinum.<sup>1</sup> The iridium must be tetravalent. Oxidizing agents must be absent.

**Sample—Platinum.** Dissolve a 0.04397 gram sample of platinum containing iridium in 3 parts of concentrated hydrochloric acid and 1 part of concentrated nitric acid. Evaporate just to dryness. Add hydrochloric acid and again evaporate just to dryness. Take up with distilled water, neutralize with ammonium hydroxide and dilute to 100 cc. This is equivalent to a solution of 1 gram of ammonium chloroplatinate per liter if the platinum were pure.

*Ammonium Chloroplatinate containing Iridium.* Dissolve 0.1 gram in distilled water and dilute to 100 cc.

**Solutions.** If the solution contains mainly platinum and iridium, dilute or concentrate to approximately the concentration of the above solutions. If the iridium is not known to be in the tetravalent condition heat to and maintain at 70 to 80°. Pass chlorine gas through the solution until oxidation is complete. Remove excess chlorine by passing air or carbon dioxide through until all excess chlorine has been removed as shown by a suitable test for oxidizing agents.

*Mixed Platinum Metals.* The method of separation of iridium from osmium, ruthenium, platinum, palladium and rhodium is given under platinum.<sup>1a</sup>

**Procedure**—Dissolve 1 gram of benzidine in 10 cc. of glacial acetic acid and 50 cc. of water. The solution should not be more than 24 hours old when used. To 10 cc. of sample solution add 2 drops of the reagent. A blue color will develop at once. If the concentration of iridium is

---

<sup>1</sup> V. G. Khlopin, *Ann. inst. platine* 1, 324-30 (1926).

<sup>1a</sup> See pp. 417-18.

igh a blue precipitate will be formed. In that case the sample must be diluted to a standard concentration with 0.1 per cent pure ammonium itable mixture

10 cc. of sample solution.

are the color of the treated sample with that of the standard atinum solution similarly treated by use of a balancing type of colorimeter. The amount of iridium present in the platinum-iridium solution is then obtained from the accompanying table.

Height of 0.1% Platinum Solution in mm.	Height of Sample Solution in mm.	Percentage of Iridium in Platinum
100	$95 \pm 1$	0.05
100	$90 \pm 1$	0.1
100	$81 \pm 1$	0.2
100	$74 \pm 1$	0.3
100	$69 \pm 1$	0.4
100	$65.5 \pm 1.5$	0.5

**Standard Platinum Solution**—Dissolve 1.0 gram of pure ammonium chloroplatinate known to be free from iridium in distilled water and dilute to 1 liter.

#### IRIDIUM IN HYDROCHLORIC ACID

The colorimetric estimation of iridium in concentrated hydrochloric acid has been discussed <sup>2</sup> but details are not well worked out. In solutions containing 0.1 gram per liter, platinum, nickel, gold and palladium give pale yellow, rhodium a rose red, cobalt a sky blue, copper, iron and osmium golden yellow, and ruthenium and iridium give brown. In the absence of rhodium, iridium can be estimated in solutions of technical platinum, matching with standard iridium and platinum solutions and considering the weight of sample.

<sup>2</sup> F. Mylius and A. Mazzucchelli, *Chem. News* 112, 90-1 (1915).

## CHAPTER XLIII

### PALLADIUM

#### PALLADIUM BY NITRIC ACID

THE yellow to brown color imparted by palladium to nitric and sulfuric acids in parting of precious metal beads is proportional to the amount of palladium present.<sup>1</sup> The method is usually applicable to solutions obtained in parting. They should not be exposed to light more than 2 days.

Ruthenium imparts a pink color in the presence of sulfuric acid. Excessive boiling with nitric acid dissolves sufficient platinum to make the procedure unreliable. In nitric acid more than ten times as much platinum as palladium must not be present as platinum imparts a color of its own which will cause the result to be high. All other metals giving colors such as copper, nickel and iron must be removed during the assay and cupellation. Gold in the bead parted must be 10 times the amount of platinum and palladium together, and the silver 3 times the combined weight of these 3. If not, add known amounts of gold and silver to the bead and recupel. The bead should be annealed before parting.

The method is ordinarily accurate to  $\pm 0.1$  mg. of palladium for each determination. For more accurate results a special assay should be run and the bead parted in nitric acid only. That reduces the possible error to estimation in only the one solution.

**Sample—Mixed Platinum Metals.** The method of separation of palladium from osmium, ruthenium, platinum, rhodium and iridium is given under platinum.<sup>1a</sup>

**Procedure—Sulfuric Acid Parting Solution.** Part the metals as usual with sulfuric acid. Pour the solution into a white porcelain dish. If a yellow color is visible it indicates the presence of more than the minimum detectable amount of palladium, 0.2 mg. Cool the acid solution

---

<sup>1</sup> F. C. Robinson, *Bull. Am. Inst. Mining Met.* 1926, No. 260.

<sup>1a</sup> See p. 417.

and pour into a Nessler tube. Dilute to 50 cc. with concentrated sulfuric acid.

In a similar tube place 45 cc. of concentrated sulfuric acid. Add standard solution of palladium in sulfuric acid until the color of the sample, as viewed lengthwise through the cylinder, is matched. Carefully dilute the duplicate with concentrated sulfuric acid, and more standard if necessary, to 50 cc. and compare horizontally as a check.

*Nitric acid Parting Solution.* The procedure is the same when examining the nitric acid parting solution except that special nitric acid and a solution of palladium in nitric acid is used in preparation of the duplicate. The nitric acid extracts of the fillet are joined as sample.

If the amount of palladium present in either the sulfuric or nitric acid parting solution exceeds 5 mg. it must be diluted and the determination carried out on an aliquot.

**Standards—Special Nitric Acid.** Boil 2 liters of 1:1 nitric acid to remove nitrous fumes. When cool add 0.8 cc. of concentrated hydrochloric acid. Mix well, add a solution of silver nitrate containing at least 1.2 grams of silver and again mix well. Let stand over night and filter. The filtrate should be clear and water white.

*Palladium in Sulfuric Acid.* Grind 0.1 gram of palladium sponge to a fine powder. Dissolve in special nitric acid, adding about 0.5 gram of powdered silver to assist in solution. Add about 10 cc. of concentrated sulfuric acid and evaporate to sulfur trioxide fumes. When cool dilute to 100 cc. with concentrated sulfuric acid. Each cc. of the resulting standard contains 1 mg. of palladium.

*Palladium in Nitric Acid.* Prepare in a similar way to the sulfuric acid standard, except that evaporation to fumes is unnecessary and special nitric acid is used in place of sulfuric acid throughout.

The standard solutions are fairly constant if exposure to strong light is avoided. A nitric acid solution of palladium lost 10 per cent of the color in 6 months. They should be checked from time to time by running determinations against known amounts of palladium.

#### PALLADIUM IN PLATINUM BY POTASSIUM IODIDE

Potassium iodide imparts a deeper brown color to a platinum solution when palladium is present than when only platinum is present. The difference is sufficient to permit the estimation of palladium.<sup>2</sup> The



amount of palladium in the platinum need not exceed 0.2 per cent. Iridium, rhodium and gold do not interfere.

**Sample**—Dissolve 0.1 gram of platinum containing palladium by the method given under the previous determination.

**Procedure**—To 10 cc. of sample add 0.1 cc. of concentrated hydrochloric acid and 1 cc. of 0.1 per cent potassium iodide solution. At the same time add the same reagents to standards of 0.1 per cent platinum solution mixed with varying amounts of 0.1 per cent palladium solution. A suitable preliminary series is 0, 0.01, 0.02, 0.03, 0.04 and 0.05 cc. of palladium solution with platinum solution to make 10 cc. For accuracy more than 10 cc. portions of the dilutions must be prepared.

The color develops fully in 30 to 40 minutes and is reasonably permanent. Compare the sample with the series of standards for estimation of the palladium content.

#### PALLADIUM BY MERCUROUS CHLORIDE

This procedure is combined with the similar method for platinum.<sup>2a</sup>

---

<sup>2a</sup> See pp. 420-1.

## CHAPTER XLIV

### POTASSIUM

#### POTASSIUM AS THE CHLOROPLATINATE BY REDUCTION WITH STANNOUS CHLORIDE

THIS method for the determination of potassium depends on the precipitation and separation of potassium as the chloroplatinate, solution of this precipitate and reduction with stannous chloride.<sup>1,1a,2</sup> The color produced is a yellow proportional in intensity to the amount of platinum present which is in turn proportional to the amount of potassium present. This method admits of estimation of amounts so small that they could hardly be detected by the usual gravimetric procedure. In the range to which the method is applied, accuracy equal to the gravimetric chloroplatinate method is obtained.<sup>3</sup> It is most suitable for determination of 1-10 p.p.m. in the solutions examined. The limitation is in the filtration and washing of such small amounts of precipitate.

**Sample—Soil.** Extract 30 grams of dry soil with 100 cc. of distilled water for one-half hour. Filter and treat the filtrate as below.

**Water, Soil Extracts, etc.**<sup>4</sup> Add 2 cc. of concentrated hydrochloric acid to an aliquot of the solution containing 1-10 mg. of potassium oxide. Evaporate to dryness and ignite at a dull red heat to destroy organic matter and volatilize ammonium salts. Take up the residue with 25 cc. of 1:3 hydrochloric acid. Heat on the steam bath and, if any insoluble residue is present, filter. Wash well and combine the filtrate and washings.

**Organic Samples.**<sup>4a</sup> When ashed with perchloric and nitric acids the resulting solution is suitable for estimation of calcium, phosphorus, magnesium and potassium.

<sup>1</sup> E. Biilmann, *Z. anal. Chem.* 39, 284 (1900).

<sup>1a</sup> L. A. Hill, *J. Am. Chem. Soc.* 25, 990-2 (1903).

<sup>2</sup> A. Nemece, *Biochem. Z.* 189, 50-6 (1927).

<sup>3</sup> V. H. Morris and R. W. Gerdel, *Plant Physiol.* 8, 315-19 (1933).

<sup>4</sup> Paul L. Gow, *Hawaiian Planters' Record* 35, 401-9 (1931).

<sup>4a</sup> For details of the method see pp. 498-9.

**Procedure**—Add 2 cc. of a 2 per cent solution of chloroplatinic acid. Evaporate to dryness on the steam bath. Take up the cooled residue with 25 cc. of 95 per cent alcohol and transfer to an inorganic filter. Wash the residue on the filter with 20 cc. of 95 per cent alcohol to remove excess chloroplatinic acid. The final drainings from the filter should be colorless. Dry the filter at 100°.

Dissolve the residue from the filter with 30 cc. of hot 1:1 hydrochloric acid, running the solution into a 100 cc. volumetric flask. Wash well with hot water. Cool. To this solution or an aliquot add 3 cc. of a solution of 75 grams of stannous chloride in 400 cc. of concentrated hydrochloric acid. A yellow to brown color will develop. After 15 minutes dilute to volume and compare with a standard developed at the same time.

**Standard**—A suitable standard is 50 cc. of a solution containing 0.0516 gram of potassium chloroplatinate per liter. This solution contains 0.01 mg. of potassium oxide per cc. A comparable standard of platinum chloride contains 0.0499 gram per liter.

#### POTASSIUM AS THE CHLOROPLATINATE BY POTASSIUM IODIDE

By treatment of the solution of potassium chloroplatinate with potassium iodide in acid solution the concentration may be estimated in quantities as low as 0.1 p.p.m.<sup>5,6</sup> Ammonia and ammonium salts must be rigidly excluded. Working with 0.16–2.81 p.p.m. the maximum error was 0.02 p.p.m. when using potassium chloroplatinate. This degree of accuracy is not attained when soil solutions or salt solutions are examined. Variations of 2 p.p.m. on a 50 p.p.m. sample are not unreasonable.

**Procedure**—*Without Alcohol.* Precipitate potassium as in the preceding method. When washing with alcohol, use absolute alcohol for the last washing. Allow the alcohol to evaporate completely before proceeding, as a trace of alcohol will affect the color with potassium iodide.

Dissolve the precipitate of potassium chloroplatinate from the filter with 10 cc. of hot water and let cool. Add 1 drop of concentrated hydrochloric acid and 0.5 cc. of 10 per cent potassium iodide solution. Mix well, let stand 4 hours and dilute to 50 cc. The rate of development of color is increased by excess potassium iodide, the presence of free acidity and by elevated temperatures. A large amount of acid is not permissible.

<sup>5</sup> F. K. Cameron and G. H. Failyer, *J. Am. Chem. Soc.* 25, 1063 (1903).

<sup>6</sup> B. V. J. Cuvelier, *Natuurw. Tijdschr.* 14, 107-10 (1932).

Compare with a standard of potassium chloroplatinate in which the color has been similarly developed.

*With Alcohol.* As an alternative all of the alcohol need not be removed and the comparison made by the yellow color which develops. The color is easier to match but the method is not as sensitive.

Precipitate and wash with alcohol as in the preceding method. Without waiting for the washed precipitate to dry, dissolve it from the filter in 10 cc. of hot water. Add 0.5 cc. of 10 per cent potassium iodide solution and 0.5 cc. of alcohol immediately. Heat until the first pink color has changed to a clear yellow. Compare with a standard treated in the same way.

The solution in which the pink color has been developed may also be converted to the yellow form and used for check comparison. After comparing in the pink form add 0.5 cc. of alcohol and heat. Conversion to the yellow color is retarded by the acid present.

#### POTASSIUM SEPARATED AS THE COBALTINITRITE

Potassium may be precipitated as the double potassium-sodium cobaltinitrite,  $K_2Na[Co(NO_2)_6]H_2O$ , separated, and redissolved. While sodium cobaltinitrite is the usual reagent, zinc cobaltinitrite has been prepared<sup>6a</sup> for use as a qualitative reagent to avoid introduction of sodium. The method is by passing oxides of nitrogen through a solution saturated with cobalt acetate and zinc acetate for 45–60 minutes. It should be useful for quantitative work when sodium estimation is to follow estimation of potassium. The solution of the potassium cobaltinitrite is susceptible to development of color by practically every method used for cobalt. In solution in hydrochloric acid a green color is produced which may be estimated directly. By addition of dimethylglyoxime and sodium sulfide a brown color suitable for estimation is obtained. Other methods depend on basic cobalt carbonate and on quantitative reactions of the nitrite radical. The loss in color of the original solution due to precipitation can also be measured.<sup>7</sup>

The structure of the precipitate has often been questioned. Under varying conditions the potassium content is reported to vary from 9.3 per cent to 17.9 per cent.<sup>7a</sup> This variability has been confirmed by

<sup>6a</sup> Jane Adams, Martha Hall and W. F. Bailey, *Ind. Eng. Chem., Anal. Ed.* 7, 310 (1935).

<sup>7</sup> E. M. Emmert, *J. Assoc. Official Agr. Chem.* 14, 573-5 (1931).

<sup>7a</sup> H. W. Louise, *Ind. Eng. Chem., Anal. Ed.* 7, 272-3 (1935).

others.<sup>7b,7c,7d,7e</sup> Provided the sample and standard are precipitated under identical conditions this is unimportant since the composition of both precipitates will be substantially identical.

**Sodium Cobaltinitrite Reagent**<sup>8,9</sup>—Dissolve 25 grams of cobalt nitrate in 50 cc. of water, and add 12.5 cc. of glacial acetic acid. Dissolve 120 grams of potassium-free sodium nitrite in 180 cc. of water, and add 210 cc. of this to the solution of cobalt nitrate. Pass a current of air through the solution until all the nitric oxide gas has been carried off. Keep on ice, and filter each time before using. The reagent may be kept a month.

A modification<sup>10</sup> requires that the solution be made up more frequently. Prepare a solution of cobalt nitrate of density 1.3. To each 100 cc. of this solution add 25 cc. of glacial acetic acid. Separately dissolve 200 grams of pure sodium nitrite in 300 cc. of water by heating and let cool. For use, mix 10 cc. of the cobalt solution and 30 cc. of the sodium nitrite solution. Remove evolved nitrogen oxide by aspirating air through for 10 to 15 minutes, with frequent shaking. Let stand for 5 hours, centrifuge and filter. This should be not over 2 to 3 days old.

**Sample—Whole Blood.** Lithium citrate in a concentration of 30 mg. per 100 cc. as an anticoagulant does not interfere. Dry 0.2 cc. of blood in a platinum dish in an air bath at 110°. Heat over a low flame until no more fumes come off. Cover and heat over a large flame, still in an air bath, until completely ashed. When cool, dissolve the ash in 0.5 cc. of water containing 1 or 2 drops of glacial acetic acid. Add 0.5 cc. of sodium cobaltinitrite reagent, dropwise, with constant shaking and let stand for 15 minutes. Transfer the precipitate to a centrifuge tube with a little water and centrifuge at 2000 r.p.m. for 5 minutes. Decant off the supernatant liquid, add 2 cc. of water, shake, and centrifuge again. Repeat the washing process 3 times. Run 1 cc. of solution containing 0.2 mg. of potassium chloride at the same time, for development as a standard. A 0.1 cc. sample and 0.1 cc. standard can also be used.<sup>9</sup>

<sup>7b</sup> G. Jandler and H. Faber, *Z. anorg. Chem.* **173**, 225 (1928).

<sup>7c</sup> F. H. Taylor, *J. Biol. Chem.* **87**, 27 (1930).

<sup>7d</sup> A. H. Lewis and F. B. Marmoy, *J. Soc. Chem. Ind.* **52**, 177T (1933).

<sup>7e</sup> Albert E. Sobel and Benjamin Kramer, *J. Biol. Chem.* **100**, 561-71 (1933).

<sup>8</sup> Benjamin Kramer and Frederick F. Tisdall, *J. Biol. Chem.* **46**, 339-49 (1921).

<sup>9</sup> Miklos Dreguss, *Biochem. Z.* **233**, 375-80 (1931).

<sup>10</sup> Ch. Zinzadze, *Chimie et Industrie* (Special No.) 841-3 (March, 1932);

*Blood Serum.*<sup>7e</sup> Put 1 cc. into a centrifuge tube and add 2 cc. of sodium cobaltinitrite reagent, dropwise, with constant stirring. After 45 minutes add 2 cc. of water, mix, and centrifuge for 15 minutes at 2000–2500 r.p.m. Decant the supernatant liquid, add 2 cc. of 30 per cent alcohol, shake, and centrifuge. Decant and repeat 3 times. Put a standard through the same treatment at the same time.

*Soil.*<sup>13,14</sup> Evaporate an aliquot of the soil extract, containing about 1 mg. of potassium oxide, nearly to dryness. Add 0.5 cc. of concentrated nitric acid and complete the evaporation. Ignite gently. Add 10 cc. of water and heat for 15 minutes. Filter and wash the paper well. Evaporate the filtrate to 1 cc. Add 1 cc. of cobaltinitrite reagent and evaporate to a thick syrup. Cool, add 5 cc. of 10 per cent acetic acid, mix well and let the solution stand for 15 minutes. Filter through asbestos on a Gooch crucible. Wash with 2 cc. of 10 per cent acetic acid. Treat a similar amount of standard in the same way at the same time.

*Solutions Containing Phosphates or Iron.*<sup>15</sup> Prepare magnesia mixture by solution of 55 grams of crystallized magnesium chloride and 70 grams of ammonium chloride in 650 cc. of water. Dilute to 1 liter with 1:3 ammonium hydroxide.

Render 10 cc. of sample solution alkaline with 1:3 ammonium hydroxide. Add 1 cc. of magnesia mixture and let stand in the cold for 24 hours. This precipitates phosphates and iron but no potassium. Heating, or too much magnesia mixture, will precipitate potassium. Centrifuge and remove the liquid. Stir the precipitate with 3 cc. of 1:3 ammonium hydroxide, centrifuge and decant. Wash once more.

Evaporate the solution and washings to dryness and heat until no more fumes are evolved. The ammonia and ammonium salts are volatilized. Dissolve the residue in 4 cc. of water and transfer to a tube for precipitation of cobaltinitrite according to other methods of preparation of sample. Wash the residue from the tube with 1 cc. of water.

*Solutions free from Phosphate and Iron.*<sup>10</sup> Transfer the sample to a pointed centrifuge tube. To the sample add 1 cc. of a solution containing at least 0.02 mg. of potassium. Add 1 cc. of cobaltinitrite reagent and let stand for at least 3 hours, or better over night. Centrifuge for 15 to 20 minutes at 2000–3000 r.p.m. Decant the upper layer. Add 2 cc. of water, rinsing the sides of the tube, centrifuge and decant. Repeat

<sup>13</sup> G. Deniges, *Compt. rend. soc. biol.* 84, 63 (1921).

<sup>14</sup> J. L. Steenkamp, *J. South African Chem. Inst.* 13, 64-70 (1930).

H. J. Hamburger, *Biochem. Z.* 71, 415-63 (1915).

2 to 3 times. At the same time prepare a standard precipitate by the same treatment.

**Procedure—Direct Development as Cobalt Chloride.**<sup>17,18</sup> Dry the precipitates at 100° for 2 to 3 hours. Cool and dissolve in 3 cc. of concentrated hydrochloric acid by warming gently. Cool and transfer to comparison tubes. Compare by dilution with absolute alcohol to prevent hydrolysis, or by balancing. The standards may be kept about 1 week. Amounts of 0.025–0.5 mg. of potassium may be estimated with an error of about 4 per cent.

**Development with Dimethylglyoxime and Sodium Sulfide.**<sup>19,20</sup> Add 2 cc. of 5:2 nitric acid to the precipitates and warm until dissolved. Put 1.5 grams of sodium acetate into each of two 50 cc. volumetric flasks. Add the solutions of potassium cobaltinitrite, rinsing out the containers and adding the rinsings. To each flask add 20 cc. of water, 5 cc. of a 1 per cent alcoholic solution of dimethylglyoxime, and 2 cc. of a 1 per cent sodium sulfide solution. After 3 minutes heat both in boiling water for 15 minutes, cool and dilute to 50 cc. Compare in a colorimeter. The procedure can be carried out with 0.1 cc. of blood. The average deviation results indicated an error of not over  $\pm 5$  per cent.

**Development as Basic Carbonate.**<sup>21</sup> Dissolve the precipitates in 2 cc. portions of 1:1 hydrochloric acid. Evaporate to dryness and dissolve in 2 cc. of water. Transfer to 10 cc. volumetric flasks and add 0.5 cc. of 3 per cent hydrogen peroxide. Dilute nearly to 10 cc. with a saturated solution of potassium or ammonium bicarbonate. Mix well and dilute to volume with water or bicarbonate solution. Mix and compare.

**Development with Salicylic Acid and  $\alpha$ -Naphthylamine.**<sup>9</sup> Mix a solution of 2.5 grams of sulfanilic acid in 750 cc. of 10 per cent acetic acid with a solution prepared by boiling 1 gram of  $\alpha$ -naphthylamine with 100 cc. of water and adding 750 cc. of 10 per cent acetic acid. This reagent should be colorless and will keep well in a dark bottle.

Add 5 cc. of the reagent to each of the cobaltinitrite precipitates. Heat for 2 minutes in boiling water. Transfer the resulting red solutions to 100 cc. flasks. Add 5 cc. of the reagent to the residue and repeat. Con-

<sup>17</sup> F. Lebermann, *Biochem. Z.* 150, 548-59 (1924).

<sup>18</sup> Maurice Delaville, *Compt. rend. soc. biol.* 101, 1082-3 (1929).

<sup>19</sup> S. Yoshimatsu, *Tôhoku J. Exptl. Med.* 8, 174 (1926).

<sup>20</sup> S. Yoshimatsu and Y. Uga, *Tôhoku J. Exptl. Med.* 19, 156-61 (1932).

<sup>21</sup> A. Blanchetière and J. M. Pirlot, *Compt. rend. soc. biol.* 101, 858-60 (1929).

tinue so long as any residue remains. Cool, dilute to the mark with cold reagent and compare.

A similar reagent <sup>23,23a</sup> consists of 5 grams of sodium naphthionate and 2.5 grams of  $\beta$ -naphthol in 500 cc. of water. After shaking and filtering, the reagent is unchanged if not exposed to light. In the light it develops a rose color.

*Development with Cysteine Hydrochloride and Hydrogen Peroxide.* Cysteine combines with cobalt to give a blue-green cobaltobiscysteinate.<sup>24</sup> This reaction is the basis of a method of estimation of the potassium combined in the cobaltinitrite.<sup>7c</sup> Dissolve the precipitates in 1 cc. portions of 1:2 hydrochloric acid and evaporate to dryness. Dissolve the residues in 5 cc. of 12.5 per cent potassium pyrophosphate solution. Add 5 cc. of a solution containing 3.5 mg. of cysteine hydrochloride per cc. Add 2.5 cc. of 0.1 per cent hydrogen peroxide solution. Dilute to 25 cc. or other suitable volume and compare.

*Development with Choline Hydrochloride and Potassium Ferrocyanide.*<sup>26</sup> Dissolve the precipitates in 2 cc. of water. Add 1 cc. of a 1 per cent solution of choline hydrochloride and 1 cc. of a 2 per cent potassium ferrocyanide solution. Dilute each to 6 cc. An emerald green color develops. Compare. The method is as accurate as those with chloroplatinate.<sup>27</sup>

*Development as Tropaeolin.*<sup>28</sup> Dissolve the precipitates in 5 cc. portions of 0.1 *N* sodium hydroxide solution by warming on a water bath. Disregard separated cobalt hydroxide. Transfer the solutions to 100 cc. volumetric flasks with about 50 cc. of water.

Prepare a reagent containing 1 gram of sulfanilic acid in 100 cc. of hot saturated ammonium chloride solution, mixed with 1.5 grams of phenol and 100 cc. of water.

Add 1 cc. of this reagent and 1 cc. of 1:3 hydrochloric acid. Mix and let stand 15 minutes. Add 1:3 ammonium hydroxide until each is distinctly alkaline, and dilute to 100 cc. with water. Compare the resulting yellow azo dye solutions.

---

<sup>23</sup> Joseph Tischer, *Biochem. Z.* 238, 148-61 (1931).

<sup>23a</sup> Cf. F. Alten and H. Weiland, *Z. Pflanzenernähr., Düngung Bodenbk.* 34A, 108-10 (1934).

<sup>24</sup> Maxwell P. Schubert, *J. Am. Chem. Soc.* 53, 3851-61 (1931).

<sup>26</sup> H. R. D. Jacobs and William S. Hoffman, *J. Biol. Chem.* 93, 685-91 (1932).

<sup>27</sup> V. H. Morris and R. W. Gerdel, *Plant Physiol.* 8, 315-19 (1933).

<sup>28</sup> S. N. Rozanov and Valentina A. Kazarinova, *Z. anal. Chem.* 96, 26-9 (1934).



*Development with Thiocyanate in Acetone.*<sup>29,30,30a</sup> Dissolve the precipitates in 0.5 cc. portions of 1:4 sulfuric acid by warming on the water bath. Transfer with 75 per cent acetone solution to 25 cc. volumetric flasks. Add 5 cc. of a 5 per cent solution of ammonium thiocyanate in acetone and dilute to volume with 75 per cent acetone solution. The resulting clear blue color is stable for several days. Compare.

By another modification,<sup>31-33</sup> the same color is developed but is extracted with ether-amyl alcohol mixture.

*Development with Antipyrine.*<sup>34</sup> Treat the precipitates with 1 cc. portions of 5 per cent sodium hydroxide solution and heat to boiling. Cobalt is precipitated as the hydroxide and the nitrite is present as sodium nitrite. Separate the precipitates by centrifuging and wash with 3 successive 1 cc. portions of water. Neutralize the solutions and wash waters with 1:10 sulfuric acid and dilute each to 7 cc. Add 3 cc. of 5 per cent antipyrine solution and 1 drop of concentrated sulfuric acid. Mix and compare the green colors of sample and standard. The method is accurate to 3 per cent.

**Standard**—Dissolve 1.907 grams of potassium chloride in water and dilute to 1 liter. Each cc. contains 1 mg. of potassium. Dilute 100 cc. of this to 1 liter to give a standard containing 0.1 mg. of potassium per cc., or 10 cc. to 1 liter to give 0.01 mg. of potassium per cc.

#### POTASSIUM AS THE PICRATE

By precipitation of potassium picrate in alcoholic solution at 20° and subsequent solution of the precipitate in water a yellow solution is obtained suitable for the colorimetric estimation of potassium.<sup>35</sup> Below 20° some picric acid is precipitated and above that temperature the solubility of potassium picrate causes low results.

Sulfates must be eliminated by precipitation with barium chloride.

<sup>29</sup> E. S. Tomula, *Acta Chem. Fennica* 2, 72-80 (1929); *Z. anal. Chem.* 83, 6-14 (1931).

<sup>30</sup> Friedrich Steffen, *Schweiz. med. Wochschr.* 62, 13-15 (1932).

<sup>30a</sup> Cf. Ch. R. Zinzadze, *Trans. Sci. Inst. Fertilizers (U. S. S. R.)*, No. 109, 91-4 (1932).

<sup>31</sup> A. D. Marenzi and R. Gerschmann, *Compt. rend. soc. biol.* 110, 145-7 (1932).

<sup>32</sup> Rebeca Gerschmann and A. D. Marenzi, *Anales farm. bioquim.* 2, 194-204 (1931).

<sup>33</sup> A. Durupt and F. Schlesinger, *Bull. soc. chim. biol.* 13, 700-21 (1931).

<sup>34</sup> M. Mousseron, *Bull. soc. chim. biol.* 13, 831-4 (1931).

<sup>35</sup> Earle R. Caley, *J. Am. Chem. Soc.* 53, 539-45 (1931).

Small amounts of insoluble matter do not interfere. Ammonium salts must be absent. Rubidium; Cesium, magnesium, aluminum, iron, phosphates not exceed 20 mg. in the sample used.

**Procedure**—Evaporate the solution containing potassium as the water bath. Dissolve the residue in 1 cc. of acid in  
of 2  
ing, 7.5 cc.

flask. After dilu  
potassium picrate  
chloride solution.<sup>36a</sup>

#### POTASSIUM AND SODIUM BY EOSIN

not given in detail.

<sup>36</sup> I. N. Antipov-Karátsev and A. M. Myasnikova, *Proc. Leningrad Dept. Inst. Fert.* 17, 81-8 (1933).

<sup>36a</sup> See p. 437.

<sup>37</sup> F. Mylius and F. Forster, *Z. anal. Chem.* 31, 241-82 (1892).

## CHAPTER XLV

### SODIUM

#### SODIUM SEPARATED AS A COMPLEX URANYL SODIUM ACETATE

UNDER proper conditions sodium can be precipitated as a complex compound of zinc or magnesium with uranyl and acetate radicals. The formula of the zinc compound is  $[\text{UO}_2(\text{CH}_3\text{COO})_2]_3 \cdot \text{Zn}(\text{CH}_3\text{COO})_2 \cdot \text{NaCH}_3\text{COO} \cdot 9\text{H}_2\text{O}$ . The precipitation is most complete in 33 per cent alcohol. The greenish color of the aqueous solution may be directly compared with that of a standard,<sup>1</sup> or the sodium indirectly estimated by conversion of the uranyl radical to uranyl potassium ferrocyanide,  $\text{UO}_2\text{K}_2\text{Fe}(\text{CN})_6$ , which has a stable brownish red color.<sup>2,3</sup> The original method of Barrenscheen and Messinger has been criticized as not giving quantitative separation.<sup>4</sup> The coloration produced is independent of the acidity between 0.04 and 5.0 per cent of acetic acid. Reaction in 2 per cent acetic acid solution has been recommended.<sup>4a</sup> The reaction does not occur in an alkaline medium. With a 0.2 per cent solution of the uranium salt a 1 per cent solution of potassium ferrocyanide gives the optimum color.<sup>5</sup> Large additions of potassium ferrocyanide increase the color and delay precipitation.<sup>6</sup> Under some conditions the color increases for 3 hours, then fades slowly. Precipitation is complete at 0–10°. The precipitate is appreciably soluble at 20° and even more so at 37°. The color is proportional to the sodium content within a range of 50 per cent variation between sample and standard. The addition of alcohol to the reagent prevents error due to sodium in the reagents.

Phosphorus would precipitate with the reagent and therefore must be removed. It can be precipitated as zinc phosphate but is more con-

<sup>1</sup> Earle R. Caley and C. W. Foulk, *J. Am. Chem. Soc.* 51, 1664-74 (1929).

<sup>2</sup> H. K. Barrenscheen and L. Messinger, *Biochem. Z.* 189, 308, 423 (1927).

<sup>3</sup> H. H. Barber and I. M. Kolthoff, *J. Am. Chem. Soc.* 50, 1625-31 (1928); *Ibid.* 51, 3233-7 (1929).

<sup>4</sup> Robert A. McCance and Henry L. Shipp, *Biochem. J.* 25, 449-56 (1931).

<sup>4a</sup> K. L. Malyarov and T. Yudenich, *Zavodskaya Lab.* 3, 904-6 (1934).

<sup>5</sup> Marguerite Tissier and Henri Bénard, *Compt. rend. soc. biol.* 99, 1144-6 (1928).

<sup>6</sup> F. Alten and H. Weiland, *Z. Pflanzenernähr., Düngung Bodenk.* 31A, 252-5 (1933).

veniently removed with hydrated lime, thus eliminating dilution by the precipitating agent. Similarly, proteins can be removed with mercuric chloride. Calcium, magnesium, strontium, barium and iron do not interfere.<sup>1</sup> Potassium must not exceed 5 times the amount of sodium unless a special procedure is followed. The optimum amount of sodium is about 0.3 mg.<sup>8</sup> The method is suitable for estimation of sodium in water.<sup>8a</sup>

**Reagent**<sup>4</sup>—Dissolve 10 grams of uranyl acetate in 50 cc. of boiling water containing 2 cc. of glacial acetic acid. Dissolve 30 grams of zinc acetate in 50 cc. of boiling water containing 1 cc. of glacial acetic acid. Mix the boiling solutions, heat again just to boiling and let stand over night. Filter and mix the filtrate with an equal volume of absolute alcohol. Let stand 48 hours at 0° and filter at 0°. The reagent is stable at room temperature.

This modification of Kolthoff's original reagent<sup>3,11</sup> is more accurate because traces of sodium present in the reagent are more efficiently precipitated.

**Sample**—*Urine, Normal Sodium Content*.<sup>12</sup> Measure 6 cc. as sample. Add 1 drop of phenolphthalein solution and 0.2 gram of hydrated lime. The latter precipitates phosphates. If proteins are present add 0.05 gram of mercuric chloride. Shake and let stand for 30 minutes with occasional shaking. The solution should turn pink with excess lime. Filter and collect the filtrate in a test tube. If protein was present, test the filtrate to insure complete precipitation, and if necessary add more mercuric chloride and filter again. Stopper the sample to prevent precipitation of calcium carbonate.

Fit a rubber stopper in the bottom of a porous glass filter of 30 cc. capacity. Pipet 20 cc. of reagent into the filter and add 2 cc. of the urine filtrate. Stir to cause precipitation and continue to stir or results will be low. When precipitation is complete rinse the rod with 3 cc. of reagent and cover. Let stand 1 hour at room temperature. Remove the stopper and apply suction. When the reagent has all gone through wash with five 2 cc. portions of 95 per cent alcohol saturated with the triple acetate. Wash down the sides of the filter with this. Then wash with two 5 cc. portions of ether and dry by drawing air through.

<sup>8</sup> Peter W. Salit, *J. Biol. Chem.* 96, 659-72 (1932).

<sup>8a</sup> O. M. Smith and Herbert Blair, *Proc. Oklahoma Aca. Sci.* 13, 33-5 (1934).

<sup>11</sup> Cf. A. Blanchetière, *Bull. soc. chim.* 33, 807-18 (1923).

<sup>12</sup> A. M. Butler and Elizabeth Tuthill, *J. Biol. Chem.* 93, 171-80 (1931).

*Urine of Minimal Sodium Content.*<sup>12</sup> Measure 50 cc. of sample into a Kjeldahl flask. Ash with nitric acid and hydrogen peroxide<sup>14</sup> and evaporate to dryness. Extract the dry residue with 10 cc. of warm water and transfer to a 25 cc. volumetric flask without filtering. Dilute to volume and pour into a small Erlenmeyer flask. Add 0.4 gram of hydrated lime, let stand 1 hour with occasional shaking and filter. Test the filtrate to insure absence of phosphate. Measure 20 cc. of filtrate and evaporate to dryness on a water bath. Add 5 cc. of hot water and 1 drop of concentrated hydrochloric acid. Shake for a few minutes and add 3 cc. of a saturated solution of ammonium perchlorate. This precipitates excess of potassium as perchlorate. Cool, let stand one-half hour to complete precipitation and filter into a beaker. Wash the precipitate with 95 per cent alcohol and evaporate the filtrate to dryness. Dissolve the residue in 2 cc. of water and proceed as for normal urines starting "Fit a rubber stopper . . .," except that only 15 cc. of reagent are used instead of 20 cc.

*Serum.*<sup>12</sup> Measure 1 cc. of serum into a Pyrex test tube. Add a crystal of quartz, 1 cc. of 4 *N* sulfuric acid and 0.5 cc. of concentrated nitric acid. Digest as for a micro Kjeldahl determination. If charring occurs add more nitric acid or hydrogen peroxide, repeating if necessary. Heat a few minutes after it is clear. When cool, add 4 to 5 drops of water and transfer quantitatively to a filter containing 15 cc. of reagent, as for urine.<sup>16</sup> Rinse the test tube with 3 portions of 0.5 cc. each of water and two 3 cc. portions of reagent. The phosphorus in serum is so small that it need not be removed.

*Ash.* Prepare a zinc acetate solution as follows. Add a slight excess of concentrated ammonium hydroxide to a hot concentrated solution of zinc sulfate. Filter on a Büchner funnel and wash with hot water. Suck as dry as possible. Add the zinc hydroxide paste in small amounts to 12.5 cc. of glacial acetic acid until it is in slight excess. Filter and wash. Dilute the filtrate and washings to 100 cc. Add 3 cc. of concentrated ammonium hydroxide and 300 cc. of 95 per cent alcohol.

Measure a portion of the solution of the ash to contain 0.04–0.16 mg. of sodium into a centrifuge tube. Dilute this to 2 cc. Add 4 cc. of the alcoholic zinc acetate solution and mix. Cover with a rubber cap and let stand 2 to 3 hours at room temperature and over night at 0°. Centrifuge and measure 3 cc. into another centrifuge tube. Add 4 cc. of uranyl

<sup>14</sup> See pp. 497-9.

<sup>16</sup> See p. 440.

zinc acetate reagent and stir until precipitated. Cover for 1 hour and centrifuge. Decant and drain by inverting on filter paper. Wipe the mouth of the tube and add 4 cc. of alcohol saturated with the triple acetate and drain.

porate a suitable sample of the extract to  
Grind the residue and extract by boiling with 10 cc. of water for 5 minutes. Filter and wash well on the filter with hot water. Dilute to 25 cc. and take an aliquot equivalent to 0.25 mg. of sodium. Evaporate this to 1 cc. Add 2 cc. of uranyl zinc acetate solution and 3 cc. of absolute alcohol. Let stand one-half hour and filter on a Gooch crucible. Wash the precipitate with 95 per cent alcohol.

**Procedure—Direct Comparison.** Add water at 60–70° in 2 cc. portions until the precipitate is dissolved, collecting the solution in a comparison tube. When cool dilute to a standard volume according to the intensity of color, such as 10 cc. or 25 cc. Compare with a standard prepared by similar treatment of 2 cc. of standard solution containing 0.05 mg. of sodium per cc. If more than 5 mg. of sodium is present in the sample taken, excessive dilution will be required to reduce the color to an intensity at which it can be compared. Under those conditions the precipitate permits of determination of sodium gravimetrically.

*Comparison as Ferrocyanide. Potassium Low.* Dissolve the precipitate in 10 cc. of water and transfer to a 25 cc. volumetric flask. For relatively large sodium contents dilute to a greater volume. In a similar flask put the precipitate from 1 cc. or more of standard sodium chloride. Add 1 drop of glacial acetic acid and 0.5 cc. of 20 per cent potassium ferrocyanide to sample and standard for every 25 cc. of final volume. to the mark, mix, and compare after 3 minutes. Color standards stable for at least 1 hour.

*Comparison as Ferrocyanide. Potassium High.* Dilute the solution of sample so that the potassium content does not exceed 0.3 mg. per cc. Add 0.5 cc. of 7 per cent potassium ferrocyanide and 1 drop of glacial acetic acid to 7.5 cc. of sample solution. Compare with similar standards. By this procedure accurate results can be obtained up to a potassium-sodium ratio of 32–1.

**Standard**—Dissolve 0.1271 gram of pure sodium chloride in water and dilute to 1 liter. Each cc. corresponds to 0.05 mg. of sodium. Add a few cc. of chloroform as a preservative.

## SODIUM SEPARATED AS PYROANTIMONATE

An indirect method for sodium is to precipitate it as pyroantimonate, dissolve the sodium pyroantimonate and determine antimony from the compound in solution as the orange colloidal sulfide.<sup>19</sup> As little as 0.1 cc. of blood serum can be used for the determination. Lithium citrate at 30 mg. per 100 cc. may be used as anticoagulant for blood.

**Sample—Blood.** Place 0.1 or 0.2 cc. of blood in a platinum dish in an oven at 110° and dry. Set this on a thin layer of sand in a porcelain or metal dish and heat with a low flame until fumes are no longer given off. Cover with a porcelain or quartz plate and heat with a full flame until completely ashed. When cool dissolve in 0.5 cc. of 0.1 *N* hydrochloric acid and render slightly alkaline by the addition of 0.1 *N* potassium hydroxide solution drop by drop.

**Serum.** Use 0.1 or 0.2 cc. as sample.

**Potassium Pyroantimonate Reagent—**Add 10 grams of powdered potassium pyroantimonate to 500 cc. of boiling water. Cool rapidly and add 15 cc. of 10 per cent sodium-free potassium hydroxide solution. Stir and filter through ash-free filter paper into a paraffined bottle. The solution keeps a month or longer.

**Procedure—**To 1 cc. of potassium pyroantimonate reagent in a centrifuge tube add the sample, and then 0.3 cc. of 95 per cent alcohol, drop by drop with stirring. Let stand 45 minutes, and centrifuge. Decant and wash the precipitate with 2 cc. of 50 per cent alcohol 3 times. Dissolve the residue in 0.5 cc. of concentrated hydrochloric acid. Transfer to a 25 cc. volumetric flask, rinsing the centrifuge tube with 1 cc. of 1:10 hydrochloric acid. Put 3 cc. of a standard solution of sodium pyroantimonate into a second 25 cc. flask. To each add 10 cc. of water, 3 cc. of a 10 per cent gelatin solution, and 2.5 cc. of a 10 per cent solution of sodium sulfide. Shake, dilute to volume and compare the two solutions in a colorimeter.

**Standard—**Add slowly 50 cc. of 2.5 per cent sodium chloride solution to 500 cc. of the potassium pyroantimonate reagent. Then add 120 cc. of 95 per cent alcohol with constant stirring. Let stand at least 2 hours, transfer to a wet pad of filter paper on a Büchner funnel and wash with

<sup>19</sup> S. Yoshimatsu, *Tôhoku J. Exptl. Med.* 8, 496 (1927).

300 cc. of 50 per cent alcohol or until the washing fails to show a color on the addition of hydrochloric acid and sodium sulfide. Dissolve 1.108 grams of the dried sodium pyroantimonate in 125 cc. of concentrated hydrochloric acid and dilute to 1 liter with water. Each cc. corresponds to 0.1 mg. of sodium.

#### SODIUM SEPARATED AS SODIUM CAESIUM BISMUTH NITRITE

Sodium may be separated from potassium and other metals with which it is commonly associated by precipitation as the complex sodium caesium bismuth nitrite,  $6\text{NaNO}_2 \cdot 9\text{CsNO}_2 \cdot 5\text{Bi}(\text{NO}_2)_3$ .<sup>20</sup> This salt may be dissolved and sodium indirectly determined by the bright red color produced by nitrites with sulfanilic acid and  $\alpha$ -naphthylamine.<sup>21</sup> Chlorides should not be present in concentrations greater than 0.2 *N* or bismuth oxychloride may separate. More than traces of phosphates should be removed. Other interfering substances are iodides, citrates, and most of the heavy metals, particularly iron and silver. The use of potassium-glass containers is recommended. Another indirect method is to estimate bismuth as the colloidal sulfide.<sup>22</sup>

**Sample**—*Blood, Plasma or Urine.* Put 1 cc. of sample into a Pyrex tube. Add a few drops of concentrated sulfuric acid and 5 cc. of concentrated nitric acid. Boil over a low flame until the liquid is colorless. This is usually 45 minutes for blood, but only about 10 minutes for urine. When cool add 5 cc. of water and transfer to a 25 cc. volumetric flask. Rinse the tube with two 5 cc. portions of water and add to the volumetric flask.

Add 1 drop of methyl orange and 5 to 6 drops of a 4 per cent solution of bismuth nitrate in 2 *N* nitric acid. Add dropwise, with shaking, a 50 per cent solution of potassium carbonate free from sodium, until the indicator changes color. Dilute to volume, mix and transfer to a centrifuge tube. Centrifuge at a moderate speed to throw down phosphates and iron salts. Pipet 20 cc. of supernatant liquid into a 50 cc. Erlenmeyer flask and evaporate to about 3 cc. A loose bulb resting on the mouth of the flask is advisable to prevent loss from bumping. Make just acid with 2 *N* nitric acid, then add 0.5 cc. of 2 *N* nitric acid in excess.

*Blood or Plasma. Alternative Method.* Transfer 5 cc. of whole blood

<sup>20</sup> W. C. Ball, *J. Chem. Soc.* 97, 1408 (1910).

<sup>21</sup> E. A. Doisy and R. D. Bell, *J. Biol. Chem.* 45, 313 (1920).

<sup>22</sup> E. Tschopp, *Helvetica Chim. Acta* 8, 893 (1925).



or plasma to a 50 cc. flask containing 35 cc. of water. Add 5 cc. of 20 per cent trichloroacetic acid and dilute to 50 cc. Mix, and filter at the end of one-half hour. Pipet a 10 cc. aliquot of the filtrate corresponding to a 1 cc. sample into a 50 cc. Erlenmeyer flask and add 1 drop of concentrated nitric acid. Heat the flask, closed with a trap, until brown fumes appear. Remove, cool, and rinse the trap into the solution with a few drops of water.

*Urine, Gall, Feces, Milk, Organs or Plant Tissue.* Boil 2 cc. or a 2 gram sample with 5 cc. of fuming nitric acid over a small flame until the acid is completely volatilized. Dissolve the residue in 2 cc. of distilled water and 1 cc. of 2 *N* nitric acid. If not completely soluble, add more nitric acid and ash again. Then dissolve in 2 cc. of distilled water and 1 cc. of 2 *N* nitric acid.

If the iron or phosphorus content is high add 1 drop of methyl orange solution and 5 drops of a 4 per cent bismuth nitrate solution. Add 50 per cent potassium carbonate solution drop by drop until the color changes. Dilute with distilled water to 10 cc. Centrifuge or allow the precipitate of iron and phosphates to settle. Pipet 5 cc. of the clear supernatant layer, corresponding to 1 cc. or 1 gram of sample.

**Reagent**—*Bismuth Caesium Nitrite Solution.* If sodium-free potassium nitrite is not available prepare it as follows. Generate nitrous fumes by dropping 1:1 nitric acid from a separatory funnel into a flask containing arsenious oxide. Conduct the nitrous fumes into a 25 per cent solution of sodium-free potassium carbonate. When the solution gives off many fine bubbles of carbon dioxide on shaking, the reaction is complete. Pure potassium nitrite is precipitated. Filter, wash with several small volumes of ice water and dry.

Dissolve 30 grams of sodium-free potassium nitrite in about 60 cc. of water. Add 3 grams of bismuth nitrate dissolved in 5 cc. of 2 *N* nitric acid. If a precipitate forms add 1:1 nitric acid until it redissolves. Add 16 cc. of a 10 per cent solution of caesium nitrate. Dilute to 100 cc. and again add nitric acid if any turbidity appears. The reagent should be a clear orange-yellow. If sodium salts were present cool to 1° and filter off the precipitate at the end of 24 hours. This reagent keeps under illuminating gas at 1° for several weeks. If exposed to the air a white scum forms.

**Procedure**—Cool the solution to 10–12° and add 3 cc. of bismuth caesium nitrite solution for each milligram of sodium expected. Stopper

the flask with a 2-holed rubber stopper holding 2 right angled glass tubes. Attach to one a short rubber tube with a glass plug, and to the other a Bunsen valve and plug. Remove the plugs, pass illuminating gas free from hydrogen sulfide into the flask for a few seconds, and replace the plugs. Put in a room at 1° for 24 hours, when a yellow crystalline precipitate will have formed. At room temperature it takes 48 hours, and a scum is much more likely to form.

*Development of Color with Sulfanilic Acid and  $\alpha$ -Naphthylamine.* Filter rapidly on a Gooch crucible. Wash quickly with five 2 cc. portions of ice cold 50 per cent acetone solution, saturated with sodium caesium bismuth nitrite. Transfer the precipitate to a beaker and dissolve by warming with 10 cc. of an alkaline tartrate mixture containing equal parts of 10 per cent potassium hydroxide solution and 10 per cent tartaric acid solution. Transfer to a 100 cc. volumetric flask, cool, dilute to volume, and mix. Remove an aliquot corresponding to about 0.01 mg. of sodium to another 100 cc. flask. Dilute this and 1 cc. of a standard nitrite solution in a similar flask to about 90 cc. Add to each 2 cc. of an 0.8 per cent solution of sulfanilic acid in 5 *N* acetic acid, and 2 cc. of a 0.5 per cent solution of  $\alpha$ -naphthylamine in 5 *N* acetic acid. Dilute to 100 cc., shake, and compare after allowing 20 minutes for development of the color. As this develops the color from the nitrite present it may be very bright. The average error is about 5 per cent.

As the standard sodium nitrite solution, dissolve 0.0300 gram of pure sodium nitrite in water and dilute to 1 liter. Each cc. contains nitrite equivalent to 0.01 mg. of sodium.

*Development of Color with Hydrogen Sulfide.*—Filter and wash the precipitate exactly as in the preceding method. Dissolve the precipitate in 10 cc. of 2 *N* nitric acid, transfer to a 100 cc. volumetric flask and dilute to volume. Transfer 10 cc. of this to a 25 cc. volumetric flask and add 5 cc. of a 1 per cent gum arabic solution. Dilute to volume with a saturated aqueous solution of hydrogen sulfide. Compare with a standard bismuth solution treated with the same reagents.

As the standard bismuth nitrate solution, dissolve 7.591 grams of pure bismuth in about 50 cc. of concentrated nitric acid and dilute to 1 liter. Dilute 10 cc. of this to 1 liter. A 10 cc. portion of the latter contains 0.7591 mg. of bismuth, which corresponds to 0.1 mg. of sodium in the complex compound.

## CHAPTER XLVI

### LITHIUM

#### LITHIUM AS THE STEARATE

LITHIUM stearate, unlike other alkali stearates, is relatively insoluble in organic solvents. This property is the basis of a method for its estimation.<sup>1</sup> The method is accurate to 0.02 mg. in the lower concentrations and to 0.05 mg. in the upper ranges.

**Sample**<sup>2</sup>—Concentrate a suitable volume of the hydrochloric acid solution of the sample to a small volume. Transfer to an Erlenmeyer flask. Add 10 cc. of amyl alcohol and heat on a sand bath until all of the water has been expelled. If necessary add more amyl alcohol. This operation will be facilitated by passing a slow stream of dry air through the solution.

The precipitate which separates is sodium and potassium chlorides. Some lithium hydroxide may also separate. Decant the solution of lithium chloride in amyl alcohol through a dry filter. Wash the residue of salts and the filter with 2–3 cc. portions of hot amyl alcohol.

Moisten the residue of salts with 2 cc. of 1:1 hydrochloric acid, dissolve in 3 cc. of water, and concentrate to a small volume. Repeat the extraction with amyl alcohol. This operation may have to be repeated 4 to 5 times if much lithium is present. Normally twice will suffice when the amount of lithium is so small as to require the colorimetric method of estimation. Combine the amyl alcohol extracts and dilute to a suitable volume for use as sample.

**Reagent**<sup>3</sup>—Dissolve about 20 grams of stearic acid in 1 liter of ether. Pass in ammonia gas until no further precipitation of ammonium stearate takes place. Add ether from time to time to replace that lost by evaporation. Pour the suspension into a large tray and let the ether evaporate.

Dissolve 2 grams of the ammonium stearate in 100 cc. of warm amyl

<sup>1</sup> Earle R. Caley, *J. Am. Chem. Soc.* 52, 2754-8 (1930).

<sup>2</sup> F. A. Gooch, *Proc. Am. Acad. Arts Sci.* 22, 177-94 (1886).

<sup>3</sup> LeRoy McMaster, *J. Am. Chem. Soc.* 36, 1918 (1914).

alcohol.

. over  $50^{\circ}$  or the ammonium stear-

ate will be

saturation.

evolves ammonia on standing.

**Procedure**—Place 2.0 cc. of the amyl alcohol solution of the sample in a test tube. Prepare others with 0.05, 0.075, 0.1, 0.15, 0.25 and 0.4 mg. of lithium in 2 cc. of amyl alcohol. To each, sample and standards, add 5.0 cc. of the ammonium stearate reagent in amyl alcohol. Stopper and shake all at the same time. Let stand for 30 minutes, shake again and compare.

## CHAPTER XLVII

### CALCIUM AND BARIUM

#### CALCIUM AS SOAP

CALCIUM may be estimated nephelometrically as an insoluble soap, calcium stearate,<sup>1</sup> calcium oleate<sup>2</sup> or calcium ricinoleate.<sup>3</sup> It is first separated from interfering substances, particularly magnesium, by precipitation as the oxalate, and redissolved in acid.

**Sample—Urine.**<sup>3</sup> If the urine is normal take 8 cc. as sample. If calcium is high take 5 cc., or if low 10 cc. Transfer to a small flask. If alkaline make slightly acid. Add concentrated ammonium hydroxide to faint alkalinity. Add concentrated hydrochloric acid to faint acidity and 1 drop in excess. Add 1 cc. of 2 per cent oxalic acid solution and 1 cc. of 10 per cent sodium acetate solution. Shake for 10 minutes and rinse off the stopper with 2 cc. of 0.5 per cent ammonium oxalate solution. Shaking eliminates standing over night for precipitation. If the precipitation is carried out in the centrifuge tube the crystalline precipitate clings to the walls of the tube and cannot be properly sedimented. Transfer to a tube and centrifuge for 2 to 3 minutes, which will give a clear upper layer. Decant and add 10 cc. of 0.5 per cent ammonium oxalate solution. Mix well, centrifuge again and decant.

**Feces.**<sup>3</sup> Weigh 5 grams into a silica dish and moisten with 2 cc. of concentrated sulfuric acid. Heat gently at first and finally more strongly until a white ash remains. Transfer the ash with 50 cc. of 1:2 hydrochloric acid to a 100 cc. volumetric flask. Dilute to volume, shake and filter. Take a 10 cc. aliquot of the filtrate, equivalent to a 1 gram sample and precipitate exactly as for urine, except that it is advisable to add 2 cc. of 10 per cent sodium acetate solution instead of only 1 cc., to prevent the precipitate from coming down in too fine a form. If necessary to allow for abnormally high or low calcium content, vary the weight of sample ashed, the dilution of the ash or the aliquot taken.

---

<sup>1</sup> H. Lyman, *J. Biol. Chem.* 29, 169-78 (1917).

<sup>2</sup> A. Gregoire, *J. Soc. Chem. Ind.* 42, 427A (1923).

<sup>3</sup> H. Lyman, *J. Biol. Chem.* 21, 551 (1915).

*Blood.*<sup>1</sup> Run 5 cc. of blood into a flask containing 15 cc. of 6.5 per cent trichloroacetic acid with vigorous shaking. Mix well, let stand 10 minutes and filter through an acid-washed paper. Pipet 10 cc. of filtrate equivalent to 2.5 cc. of blood into a 50 cc. flask. Add a drop of 0.1 per cent methyl orange solution and 1:10 ammonium hydroxide until just yellow. Make just acid with 0.05 *N* nitric acid and add 1 cc. in excess. Add 2 cc. of 2 per cent oxalic acid solution and 2 cc. of 10 per cent sodium acetate solution, drop by drop with vigorous mixing. Cool in tap water until a cloud appears and shake 10 minutes, or preferably, let stand over night at room temperature.

Transfer to a centrifuge tube and rinse the flask into the tube with 5 cc. of 0.5 per cent ammonium oxalate solution. Centrifuge and decant the clear upper layer. Add 5 cc. of 0.5 per cent ammonium oxalate solution, mix well and centrifuge again. Decant.

*Milk.*<sup>7</sup> Pipet 5 cc. of cow's milk or 10 cc. of human milk into a 100 cc. volumetric flask and dilute to volume. Mix thoroughly. Pipet 10 cc., equivalent to 0.5 or 1 cc. respectively, and treat exactly as for blood.

*Organic Samples.*<sup>7a</sup> These may be ashed with nitric and perchloric acids. The resulting solution<sup>7b</sup> is suitable for estimation of calcium, magnesium, potassium and phosphorus.

### Determination as Stearate

**Solution of Sample—*Urine or Feces.*** Dissolve the precipitate of calcium oxalate in 5 cc. of *N* nitric acid. Warm on a water bath if necessary. Transfer to the original flask and rinse the centrifuge tube with 5 cc. of distilled water. Agitate to dissolve any precipitate on the wall of the flask. Transfer to a volumetric flask and dilute to 100 cc. with water. Pipet 10 cc. as a sample, equivalent to one-tenth of the original sample.

***Blood or Milk.*** Dissolve the precipitate of calcium oxalate in 5 cc. of 0.1 *N* nitric acid and transfer to the original flask. Shake to dissolve any precipitate from the walls of the flask. Rinse the centrifuge tube and stirring rod with 5 cc. of water.

**Reagent**—Dissolve 4 grams of stearic acid and 0.5 cc. of oleic acid in 400 cc. of hot 95 per cent alcohol. Add 20 grams of ammonium carbonate

<sup>7</sup> George P. Sanders, *J. Biol. Chem.* **90**, 747-56 (1931).

<sup>7a</sup> H. W. Gerritz, *Ind. Eng. Chem., Anal. Ed.* **7**, 167-8 (1935).

<sup>7b</sup> For details of the method see pp. 498-9.

dissolved in 100 cc. of hot water. Boil a few minutes. When cool, add 400 cc. of 95 per cent alcohol, 100 cc. of water, and 2 cc. of concentrated ammonium hydroxide. Filter. The solution should be water-clear and colorless. To test the reagent, pipet into flasks 10 cc. and 5 cc. respectively of calcium oxalate standard. To the 5 cc. portion add 5 cc. of 0.05 *N* nitric acid. Treat each with 25 cc. of ammonium stearate reagent and see if one gives twice the reading of the other.

**Procedure**—Into another flask pipet 10 cc. of standard calcium oxalate solution containing 0.02 mg. of calcium per cc. in 0.05 *N* nitric acid. Pipet 25 cc. of ammonium stearate reagent into two dry beakers. Pour the standard solution into one and pour back into the flask, repeating twice. Do the same with the sample. Stopper both, shake well and let stand for 10 minutes. Compare nephelometrically. Check the nephelometer by use of the same solution in both tubes.

**Standard**—Dissolve 0.0729 gram of pure calcium oxalate monohydrate in 25 cc. of 2 *N* nitric acid and dilute to 1 liter. Each cc. corresponds to 0.02 mg. of calcium in 0.05 *N* nitric acid.

### Determination as Ricinoleate

**Solution of Sample**—*Urine or Feces*. Dissolve the precipitated calcium oxalate in 5 cc. of 1:5 hydrochloric acid. Warm on a water bath if necessary to obtain the solution. Transfer to the original flask, rinse the centrifuge tube and stirring rod with 5 cc. of distilled water and agitate to dissolve any precipitate from the side of the flask.

*Blood or Milk*. Precipitate 5 times as much as specified for determination as the stearate. Treat the precipitate as above for urine or feces.

**Reagent**—Prepare the reagent by saponification of castor oil. Dissolve 15 grams of potassium hydroxide in 125 cc. of 80 per cent alcohol. Warm and add 100 cc. of castor oil. Reflux on a boiling water bath until a sample dissolves completely in water, without separation of free oil. About 7 hours refluxing is usually required. This is the stock for preparation of the diluted reagent.

Pipet 35 cc. of the castor oil soap solution into a 1 liter flask and add 9 grams of sodium hydroxide dissolved in about 500 cc. of water. Dilute to volume and mix well. The reagent should be perfectly clear and nearly colorless. This dilute reagent keeps about a week.

**Procedure**—In another flask similar to that containing the prepared sample take 10 cc. of standard calcium oxalate solution containing 0.15 mg. of calcium per cc. To sample and standard add 20 cc. of potassium ricinoleate reagent and mix well by shaking. Let stand 2 minutes and compare nephelometrically. The cloud increases for 15 to 20 minutes but if standard and sample are similar in composition this will be parallel. The sample should show on determination not less than 0.75 nor more than 2.5 mg. of calcium. The developed sample cannot be diluted.

**Standard**—Dissolve 0.5475 gram of calcium oxalate monohydrate in about 500 cc. of 1:10 hydrochloric acid and dilute to 1 liter with the same concentration of acid. This contains 0.15 mg. of calcium per cc.

### Determination as Oleate

**Sample**<sup>8</sup>—Prepare according to the method of determination as stearate but use an aliquot only half as great as specified there. Thus the sample will ordinarily be only 5 cc.

**Reagent**—Dissolve 2 grams of oleic acid and 0.5 gram of potassium hydroxide in 600 cc. of 95 per cent alcohol and dilute with alcohol to 1 liter.

**Procedure**—Dilute the sample in a 50 cc. volumetric flask to about 45 cc. In another similar flask place 5 cc. of the standard calcium solution prepared as for use in determination as the stearate<sup>9</sup> and dilute to about 45 cc.

To each add 1 cc. of a solution containing 20 per cent of sodium potassium tartrate and 7.5 per cent of potassium hydroxide. Mix well, add 1 cc. of the reagent to each and mix again. Dilute to 50 cc. and mix a third time. Compare nephelometrically after 1 hour, preferably with a series of standards.

The amount of calcium present may be too great for the procedure in which case the aliquot of the sample, the standard, or both should be reduced. The original reference includes a method for detection of traces of calcium in distilled water and a formula for estimation of errors when the calcium content is very small.

<sup>8</sup> A. Gregoire, E. Carpiaux, E. Larose and T. Sola, *Bull. soc. chim. Belg.* 32, 123-30 (1923).

<sup>9</sup> See p. 451.



## CALCIUM BY SODIUM SULFORICINOLEATE

Calcium may be determined by the turbidity produced with sodium sulforicinoate. Magnesium does not produce turbidity in the presence of ammonium ion.<sup>10</sup> The originators<sup>11</sup> reported the method to include both calcium and magnesium. Although the method is closely related to determination of calcium as a ricinoate soap it is given separately because calcium need not first be separated from magnesium. This method is suitable for determination of calcium to subtract from the value for calcium and magnesium obtained by the ferrocyanide method.

**Procedure**—Dissolve 10 grams of anhydrous sodium sulforicinoate in 112 cc. of *N* sodium hydroxide, dilute to 125 cc. and mix well. To 10 cc. of sample and 10 cc. of standard calcium solution containing 0.02 mg. of calcium per cc.<sup>12</sup> add 1 cc. of 10 per cent ammonium chloride and 0.4 cc. of sodium sulforicinoate solution. Mix. After 3 minutes dilute to 25 cc., mix, and compare nephelometrically at once.

## CALCIUM AND MAGNESIUM AS THE FERROCYANIDE

Calcium and magnesium in blood or water may be determined together by precipitating as the ferrocyanide in a 1:1 alcoholic mixture.<sup>13,10</sup> A green turbidity is produced. Barium and strontium react only in concentrated solutions of their salts. The variation from gravimetric methods is not over 3 per cent. About 1 p.p.m. can be detected.

**Sample—Blood.** Dry 1 cc. of blood in a platinum dish and ash below redness in an electric oven. Take up with 1 cc. of 1:3 hydrochloric acid. Dilute with distilled water to 15 cc., warm, and make faintly alkaline with 1:10 ammonium hydroxide. Filter off iron, if present, collecting the filtrate in a 25 cc. volumetric flask. Wash the precipitate and dilute to 25 cc. To 10 cc. of the above solution add 10 cc. of 95 per cent alcohol and mix.

**Tissue.** Ash 1 gram in the same way as for blood and follow the same procedure, except that ammonium acetate is added instead of ammonium hydroxide.

**Water.** Take 10 cc. or less according to the mineral content. If less

<sup>10</sup> L. Kriss, *Biochem. Z.* 158, 203-4 (1925); *Ibid.* 162, 359-65 (1925).

<sup>11</sup> P. Rona and H. Kleinmann, *Biochem. Z.* 137, 157 (1923).

<sup>12</sup> See p. 454.

<sup>13</sup> F. Feigl and F. Pavelka, *Mikrochemie* 2, 285-91 (1924).

than 10 cc. is used dilute to 10 cc. with distilled water. If carbonate hardness is high acidify with a drop of concentrated hydrochloric acid, boil, cool and bring back to neutrality with 1:10 ammonium hydroxide. Add 10 cc. of 95 per cent alcohol and mix.

**Procedure**—To 10 cc. of standard calcium solution containing 1 mg. of calcium oxide per cc. add 10 cc. of 95 per cent alcohol and mix. To sample and standard add 5 cc. of fresh 1 per cent ammonium ferrocyanide solution. Mix well and compare nephelometrically. The value obtained is the sum of calcium and magnesium reported as calcium. For calcium alone a prior separation of magnesium must be carried out. The ratio of concentration of sample and standard must not differ by more than 2:1.

**Standard**—Dissolve 1.785 grams of calcium carbonate in 50 cc. of N hydrochloric acid and dilute with water to 1 liter. Each cc. contains 1 mg. of calcium oxide. Dilute 100 cc. of this to 1 liter to give a standard containing 0.1 mg. of calcium per cc., 20 cc. to 1 liter to give 0.02 mg. per cc., or 10 cc. to 1 liter to give 0.01 mg. per cc.

#### CALCIUM BY REDUCTION OF THE PHOSPHATE WITH HYDROQUINONE

Either calcium or magnesium may be determined indirectly as the phosphate, with the subsequent formation of a phosphomolybdate and reduction to "molybdenum blue."<sup>15,16</sup> Calcium is separated first as the oxalate. Arsenic must be absent, as it is determined by a similar method. It has been stated that the method often gives low results.<sup>10</sup>

**Sample**—The sample should contain about 0.2 mg. of calcium in a volume of 10 cc. For blood dilute a 4 cc. sample with 8 cc. of water and 8 cc. of 20 per cent trichloroacetic acid. Mix well and filter. Transfer 10 cc. as sample.

**Procedure**—Place the 10 cc. sample in a test tube marked at 15 cc. and add a drop of methyl red solution. Add 1:3 ammonium hydroxide with stirring until the color changes to yellow, then a few drops of 5 per cent acetic acid to produce a faint reddish color. Add 1 cc. of a 4 per cent solution of ammonium oxalate and rub the sides with a rubber-tipped rod until a precipitate forms. Rinse off the rod and let the mixture

<sup>15</sup> A. P. Briggs, *J. Biol. Chem.* 59, 255 (1924).

<sup>16</sup> Cf. Carl Urbach, *Biochem. Z.* 241, 226-7 (1931).

stand 2 hours for complete precipitation. Centrifuge for 10 minutes at 1500 r.p.m. Decant the upper layer and save for determination of magnesium. Wash the precipitate with 5 cc. of 0.5 per cent ammonium oxalate solution and centrifuge again. Decant the washings and join with the solution for magnesium.

To the residue of calcium oxalate add 1 drop of concentrated hydrochloric acid and 0.5 cc. of 30 per cent hydrogen peroxide. Cover and heat for 30 minutes in a boiling water bath. Add 0.5 cc. of a 2 per cent solution of monopotassium phosphate and 3 drops of concentrated ammonium hydroxide. Let stand for 30 minutes to allow the calcium phosphate to precipitate, and add 20 cc. of a solution containing 200 cc. of 95 per cent alcohol and 50 cc. of concentrated ammonium hydroxide per liter. This precipitates the calcium phosphate more completely, as it is less soluble in the alcoholic mixture. Centrifuge for 10 minutes and decant off the liquid. Add another 20 cc. portion of the above alcoholic mixture, stir, rubbing the sides of the tube with a policeman, again centrifuge and decant off the liquid. To the residue of calcium phosphate add 5 cc. of water, 1 cc. of a 5 per cent ammonium molybdate solution, and 1 cc. of a reagent containing 30 grams of sodium bisulfite and 1 gram of hydroquinone in 200 cc. of phosphorus-free water.<sup>18</sup> Dilute to 15 cc. and at the end of half an hour compare with 5 cc. of a standard monopotassium phosphate solution corresponding to 0.1 mg. of phosphorus per cc. Each cc. is equivalent to 1.291 mg. of calcium.

**Standard**—Dissolve 0.4380 gram of potassium dihydrogen phosphate in water and dilute to 1 liter. Each cc. contains 0.1 mg. of phosphorus.

#### CALCIUM BY REDUCTION OF THE PHOSPHATE WITH 1, 2, 4-AMINONAPHTHOL SULFONIC ACID

This method is similar to that immediately preceding. It differs in that a simpler method of separation of calcium is used and a reducing agent is used which gives a lighter blue color.<sup>19,20</sup>

**Sample—Blood.** To 8 parts of 10 per cent trichloroacetic acid add 2 cc. of blood serum. Mix well and filter. To 5 cc. of filtrate in a centri-

<sup>18</sup> J. H. Roe and B. S. Kahn, *J. Biol. Chem.* 67, 585 (1926).

<sup>19</sup> J. H. Roe and B. S. Kahn, *J. Biol. Chem.* 81, 1-8 (1929).

<sup>20</sup> Cf. E. M. Emmert, *Plant Physiol.* 8, 469-73 (1933).

fuge tube add 1 cc. of 25 per cent sodium hydroxide solution, mix and let stand for 5 minutes. If the magnesium content is unusually large a precipitate will form at this point. In that case the methods of separation of calcium from magnesium given under previous methods should be used. Add 1 cc. of 5 per cent trisodium phosphate solution, mix well and set aside for 1 hour. Centrifuge for 2 minutes at 2000-2500 r.p.m. Decant and drain well, wiping off the edge of the tube.

In a 100 cc. graduated cylinder mix 58 cc. of 95 per cent alcohol and 10 cc. of amyl alcohol. Dilute to 100 cc. with water and add 2 drops of 1 per cent phenolphthalein solution. Add 5 per cent sodium hydroxide solution, drop by drop, until neutral. This will usually require 2-3 drops. Add 5 cc. of the neutralized alcoholic wash solution to the precipitate in the centrifuge tube and mix well with a glass rod. Centrifuge and decant as before. Dry the edge of the tube and add 1 cc. of acid molybdate containing 6.25 per cent of ammonium molybdate in 1:4 sulfuric acid. The precipitate will completely dissolve. Add 10 cc. of distilled water and mix.

**Reagent**—Dissolve 30 grams of sodium bisulfite and 1 gram of sodium sulfite in 200 cc. of water. Add 0.5 gram of pure 1, 2, 4-aminonaphthol sulfonic acid, mix well and filter. Keep in a dark bottle and renew every 2 weeks.

**Procedure**—At the same time as the sample is being treated with ammonium molybdate, similarly treat 10 cc. of a solution containing monopotassium phosphate equivalent to 0.01 mg. of calcium per cc. To sample and standard add 5 cc. of the reagent and dilute each to 15 cc. Mix well. Compare after 10 minutes.

**Standard**—Dissolve 2.265 gram of pure monopotassium phosphate in water and dilute to 1 liter. Add chloroform as a preservative. Each cc. is equivalent to 1 mg. of calcium. Pipet 10 cc. into a 1 liter flask and dilute to volume. Each cc. is equivalent to 0.01 mg. of calcium.

#### CALCIUM BY REDUCTION OF THE PHOSPHATE WITH STANNOUS CHLORIDE

This procedure, the oldest for reduction of the phosphomolybdate to molybdenum blue, has been stated to be the best.<sup>21</sup>

<sup>21</sup> T. Kuttner and H. R. Cohen, *J. Biol. Chem.* 75, 517-31 (1927).

**Procedure**—Follow the preceding method with 1, 2, 4-aminonaphthol sulfonic acid, making the following changes.

1. Omit the use of phenolphthalein for neutralization.
2. Use the alkaline phosphate solution<sup>22</sup> described in the method following this instead of the monopotassium phosphate solution for precipitation.
3. As reducing agent use 0.5 cc. of the diluted stannous chloride reagent described in the method which follows,<sup>23</sup> instead of 5 cc. of 1, 2, 4-aminonaphthol sulfonic acid.

#### MICRO ESTIMATION OF CALCIUM AS THE PHOSPHATE BY REDUCTION WITH STANNOUS CHLORIDE

The method of Roe and Kahn<sup>18</sup> has been modified somewhat and also adapted to the micro determination of calcium and phosphorus.<sup>21</sup>

**Alkaline Sodium Phosphate Mixture**—Dissolve 1 gram of trisodium phosphate in 50 cc. of water and mix with 50 cc. of a 20 per cent sodium hydroxide solution. If a precipitate forms let it settle for 24 hours, or centrifuge after 1 hour.

**Sample**—Transfer 0.1 cc. of pus or blood plasma with a 0.2 cc. pipet graduated in hundredths, to a small platinum dish. Rinse the pipet several times and add the rinsings to the dish. Cautiously evaporate to dryness, and burn off all organic matter. Add a drop or two of concentrated nitric acid and again ignite. Be careful to avoid spattering. This step may be carried out in about 10 minutes. Cool, dissolve the precipitate with successive small portions of 7 per cent trichloroacetic acid, rinsing down the sides of the dish. Transfer quantitatively to a narrow test tube with the aid of a pipet with a very fine bore. The total volume should not be much over 1 cc. Add 0.2 cc. of alkaline sodium phosphate mixture and let stand an hour. Centrifuge for 3 minutes at a high speed and discard the supernatant fluid. Let drain 1 or 2 minutes and wipe off the rim of the tube. Wash twice with 1 cc. portions of faintly alkaline 50 per cent alcohol. Drain and wipe off the rim of the tube each time. It is rather difficult to see the slight precipitate.

**Stannous Chloride Reagent**—This is prepared from a solution con-

<sup>22</sup> See below.

<sup>23</sup> See pp. 457-8.

taining 10 grams of tin in 25 cc. of concentrated hydrochloric acid. For use dilute 0.5 cc. of this with distilled water to 100 cc.

**Procedure**—To this precipitate, and to each of 2 standard solutions, one containing the equivalent of 0.01 mg. of calcium, and the other the equivalent of 0.005 mg. of calcium, add 0.4 cc. of a 1.875 per cent solution of sodium molybdate in 2.5 *N* sulfuric acid. Add 0.5 cc. of distilled water and 0.1 cc. of stannous chloride reagent. Close the tubes with rubber stoppers and invert at once. After 1 minute compare in a micro colorimeter.<sup>26</sup>

**Standard**—Dissolve 0.4394 gram of dried monopotassium phosphate in water, and dilute to 1 liter. Add a few drops of chloroform to prevent the formation of mold. Dilute 51.7 cc. to 1 liter. Each cc. corresponds to 0.01 mg. of calcium.

#### CALCIUM AS THE OXALATE BY FERRIC THIOCYANATE

Calcium, as oxalate, may be determined by the property of oxalates of decolorizing ferric thiocyanate solutions.<sup>27</sup> As phosphates possess the same property they must be absent. Magnesium does not interfere in the presence of ammonium ion. Very small amounts of calcium, as in water or blood, may be estimated by this method. Results are ordinarily accurate to 2 per cent.

**Sample**—*Blood*. Measure 2 cc. of clear serum into a 50 cc. beaker containing 10 cc. of concentrated nitric acid. Heat just below boiling for 2 to 3 hours. Increase the heat and evaporate to about 0.5 cc. Avoid spattering and do not evaporate to dryness. If charring occurs add 10 cc. more of concentrated nitric acid and repeat the evaporation. Wash down the sides of the beaker and add a drop of 1 per cent phenolphthalein solution. Add 1:3 ammonium hydroxide until slightly alkaline. Heat to boiling 2 to 3 minutes to remove excess ammonia.

Add to the hot solution, drop by drop, 1 cc. of a 1.25 per cent oxalic acid solution in 0.25 *N* hydrochloric acid, with stirring. Let cool and add 0.5 cc. of 35 per cent crystallized sodium acetate solution. After standing over night a granular precipitate of calcium oxalate is obtained.

If magnesium is to be determined in the separated solution, transfer to a centrifuge tube and centrifuge for 10 minutes. Separate the clear

<sup>26</sup> T. Kuttner, *J. Am. Med. Assoc.* 65, 245 (1915).

<sup>27</sup> W. M. Marriott and J. Howland, *J. Biol. Chem.* 32, 233 (1917).

upper layer and save. Dissolve the calcium oxalate in 0.5 cc. of concentrated nitric acid. Add 10 cc. of water and immerse in a boiling water bath. Stir by blowing air through, transfer to a beaker, evaporate to 0.5 cc. and precipitate calcium as before. If magnesium is not to be determined this second precipitation is not necessary.

Prepare a Gooch crucible with alternate layers of filter paper and asbestos. Wash the precipitate of calcium oxalate into the crucible, wash several times with 5 cc. portions of 1 per cent ammonium hydroxide, then once with alcohol made just alkaline with ammonium hydroxide, and once with ether. Put the Gooch crucible into a beaker and pour 10 cc. of 0.2 *N* hydrochloric acid into it. Let stand for several hours in a covered dish over water to prevent evaporation. Stir up the asbestos in the acid, transfer to a tube and centrifuge.

*Water and other Solutions.*—Take a sample varying from 50 cc. down to 1 cc. or even less according to the expected calcium content. Treat as above for blood.

**Reagent**—To prepare the ferric thiocyanate solution mix 5 cc. of a 0.3 per cent ammonium thiocyanate solution with 5 cc. of a 0.3 per cent ferric chloride solution, adding a few drops of 1:1 hydrochloric acid, if necessary, to clarify the liquid, and dilute to 25 cc. Let stand one-half hour before use.

**Procedure**—Transfer a 5 cc. aliquot of the clear supernatant liquid to a small tube. Add exactly 2 cc. of the prepared ferric thiocyanate solution and dilute with 0.2 *N* hydrochloric acid to 10 cc. Compare lengthwise with a series of standard calcium oxalate solutions treated in the same manner in tubes of the same size. The color will vary inversely with the calcium content.

**Standard**—Dissolve 0.0630 gram of pure hydrated oxalic acid in water. Add 200 cc. of *N* hydrochloric acid and a solution containing 0.0554 gram of anhydrous calcium chloride. Dilute to 1 liter. Each cc. corresponds to 0.02 mg. of calcium.

#### CALCIUM BY THE PHENOLIC PROPERTIES OF THE 8-HYDROXYQUINOLINE COMPLEX

Calcium is precipitated from solutions free from protein as a calcium-hydroxyquinoline complex by addition of 8-hydroxyquinoline.<sup>28</sup> By

<sup>28</sup> Shun-ichi Yoshimatsu, *Tôhoku J. Exptl. Med.* 15, 355-62 (1930).

treatment with ammonium chloride solution the calcium complex is dissolved and the corresponding magnesium precipitate is not. The calcium is estimated indirectly by the use of Folin's reagent. Results were accurate to 2 per cent. A high magnesium content does not lower the accuracy of the method.

Unsatisfactory results have been reported for the corresponding magnesium determination if the hydroxyquinoline reagent is not freshly prepared and all reagents are not negative to the starch-iodide test.<sup>29</sup> These precautions should be observed.

**Sample—Blood.** Pipet 0.1 cc. into 0.7 cc. of distilled water in a 5 cc. conical tube and mix to luke the blood. Add 0.1 cc. of 10 per cent sodium tungstate solution. Mix and add 0.1 cc. of 0.67 *N* sulfuric acid. Mix well with a glass rod. After 15 minutes filter through an inorganic filter into another centrifuge tube. Wash the first centrifuge tube and the precipitate on the filter 3 times with 0.3 cc. portions of distilled water and collect the washings in the second centrifuge tube. This contains the calcium and magnesium.

Add 0.15 cc. of a solution of 50 grams of sodium potassium tartrate in 100 cc. of distilled water. Add 0.25 cc. of *N* sodium hydroxide solution and mix. Add 0.15 cc. of a 5 per cent alcoholic 8-hydroxyquinoline solution drop by drop. Mix and after 10 minutes stir vigorously with a glass rod. After 2 minutes, when the maximum turbidity has been reached, place in a boiling water bath for 2 minutes. Cool to room temperature and let stand for 2 minutes to complete the calcium precipitation. Centrifuge, decant and wash 4 times with 1 cc. portions of the alkaline tartrate solution and 1 drop of *N* sodium hydroxide solution.

Decant and add 1 cc. of a solution containing 34 cc. of concentrated ammonium hydroxide in 1 liter of 5 per cent ammonium chloride solution. Mix well and place the tube in a water bath at 80°. Gradually raise the temperature to boiling and maintain for 1 minute. Add 1 drop of concentrated ammonium hydroxide and mix. After 3 minutes add another drop of concentrated ammonium hydroxide and mix. Filter while hot through an inorganic filter into a 10 cc. volumetric flask. Wash the precipitate 3 times with 0.5 cc. portions of distilled water. Add 0.5 cc. of 0.01 *N* hydrochloric acid to the solution and washings. In another flask take 2 cc. of standard calcium solution containing 0.01 mg. of calcium per cc. Add 1 drop of concentrated ammonium hydroxide and a few cc. of distilled water.

<sup>29</sup> F. Eichholtz and R. Berg, *Biochem. Z.* 225, 352-7 (1930).



*Blood. Alternative Sample.* Use 1 cc. of Folin-Wu<sup>30</sup> blood filtrate<sup>31</sup> as sample. Treat as above starting with "Add 0.15 cc. of a solution of 50 grams of sodium potassium tartrate. . . ."

**Phenol Reagent**—Boil 100 grams of sodium tungstate, 20 grams of phosphomolybdic acid and 50 cc. of 85 per cent phosphoric acid with 750 cc. of water in a 1500 cc. flask for 2 hours. Dilute to 1 liter with distilled water.

**Procedure**—To each flask add 1.2 cc. of 20 per cent sodium carbonate solution and mix. Add 1 cc. of phenol reagent and mix. Place the flasks in a boiling water bath immersed to half the depth of the solution and heat for 5 minutes. Dilute to the mark and mix. Let cool, again dilute to the mark and mix. Compare.

**Standard**—Add 5 grams of sodium potassium tartrate to 100 cc. of 1 per cent calcium chloride solution. Add 25 cc. of *N* sodium hydroxide solution and mix well. Add sufficient 5 per cent alcoholic 8-hydroxyquinoline solution for complete precipitation and mix well. After 0.5 hour filter on a Büchner funnel and wash several times with a solution containing 50 grams of sodium potassium tartrate, 1 cc. of concentrated ammonium hydroxide and 100 cc. of water. Dry at 120°. Dissolve 0.410 gram of the calcium-hydroxyquinoline complex so prepared in 50 cc. of *N* hydrochloric acid and dilute to 500 cc. Pipet 25 cc. of this stock solution into a 500 cc. volumetric flask and dilute to volume with distilled water. Each cc. contains 0.01 mg. of calcium.

#### CALCIUM BY SODIUM TUNGSTATE AND TITANOUS CHLORIDE

Calcium may be precipitated quantitatively by sodium tungstate. The reagent also precipitates barium, strontium, aluminum, zinc, lead, mercury and copper. Iron is also precipitated but is removed in the procedure. The tungsten may then be estimated by titanous chloride as a measure of the calcium originally present.<sup>32</sup>

**Sample**—*Serum.* Evaporate 5 cc. to dryness in a platinum dish. Ash just below redness. Extract the ash with 5 cc. of hot water and

<sup>30</sup> Otto Folin and Hsein Wu, *J. Biol. Chem.* 28, 81 (1919).

<sup>31</sup> See p. 534.

<sup>32</sup> M. Mousseron and N. Bouisson, *Bull. soc. chim. biol.* 12, 482-90 (1930).

again ash the residue. Dissolve the carbon-free ash in 2 cc. of *N* hydrochloric acid and join with the aqueous extract. Transfer to a centrifuge tube and add saturated ammonium carbonate solution to faint alkalinity. Add 2 drops of 1:1 hydrochloric acid, or more if necessary to get a clear solution. Add 5 cc. of a 5 per cent solution of sodium tungstate, being careful not to scratch the sides of the tube in mixing. Heat in a water bath at 70–80° for 1 hour. Centrifuge and decant. Wash the precipitate twice with 5 cc. of distilled water, stirring and centrifuging each time. If the second wash-water gives a test for tungstate with titanous chloride, wash with water until this test is negative. The precipitate is calcium in the form of the tungstate.

Dissolve the precipitate in 0.5 cc. of 1:3 hydrochloric acid. Heat in a water bath for 15 minutes to precipitate yellow anhydrous tungstic acid. Centrifuge and wash the precipitate with 2 cc. of *N* hydrochloric acid to remove calcium. Discard the washings. Dissolve the precipitate of tungstic acid in 2 cc. of 20 per cent potassium hydroxide solution. Neutralize to litmus with 1:1 hydrochloric acid. Add 0.3 cc. of *N* hydrochloric acid and dilute to 10 cc. with distilled water.

**Reagent**—Dilute 10 cc. of commercial 12 per cent titanous chloride to 1 liter. Standardize by titration of a solution of ferric iron with potassium thiocyanate as indicator and dilute so that each cc. is equivalent to 2 mg. of iron.

**Procedure**—Add 0.3 cc. of a titanous chloride solution, each cc. of which is equivalent to 2 mg. of iron. Compare the blue color which develops at once with that developed from suitable volumes of a standard sodium tungstate solution diluted to 10 cc.

**Standard**—Dissolve 4.050 grams of sodium tungstate in water and dilute to 1 liter. Each cc. is equivalent to 0.5 mg. of calcium.

#### CALCIUM AS THE NICKEL NITRITE COMPLEX BY ANTIPYRINE

Calcium can be quantitatively precipitated with nickel nitrite as a complex nitrite,  $K_2CaNi(NO_2)_6$ .<sup>33</sup> An indirect method is then to estimate the nitrite content of the precipitate by its reaction with antipyrine.<sup>34</sup> This reaction is sensitive to 0.1 mg. of nitrite per liter of water.

<sup>33</sup> M. Mousseron, *Bull. soc. chim. biol.* 12, 1014-21 (1930).

<sup>34</sup> M. Mousseron, *Bull. soc. chim. biol.* 13, 831-4 (1931).

The reaction is believed to be specific for the alkaline earths.<sup>35</sup> Copper, manganese, aluminum, iron, zinc and magnesium do not precipitate. The precipitate is washed with 20 per cent acetone, which introduces an error, as the compound is soluble to the extent of 0.02 mg. of calcium per cc. Accuracy to 4 per cent is obtainable. The method is much more specific than the sodium tungstate precipitation.

**Reagent**<sup>33</sup>—Dissolve 10 grams of potassium nitrite in 100 cc. of 30 per cent solution of nickel nitrate. Add 10 cc. of glacial acetic acid and let stand. Separate any cobalt present as impurity by precipitation in the form of potassium cobaltinitrite<sup>37</sup> and filtration. Distil the acid solution *in vacuo* at 60° to about 50 cc. Repeat with 3 successive additions of 50 cc. portions of water. The acetic acid will all have been removed. Dilute the neutral liquid to 100 cc. Add 45 grams of pure potassium nitrite and let stand 24 hours to precipitate traces of calcium. Decant or filter.

**Procedure**—Transfer 5 cc. of neutral sample containing about 0.5 mg. of calcium as the chloride into a centrifuge tube. Add 5 cc. of the reagent and let stand for 5 hours. Centrifuge the yellow precipitate and decant. Wash the precipitate twice with 1 cc. portions of 20 per cent acetone in water. Wash once with 2 cc. of 1:1 alcohol-ether mixture. Dissolve the nickel nitrite compound in 7 cc. of water. Add 3 cc. of a 5 per cent solution of antipyrine and 1 drop of concentrated sulfuric acid. Compare the green color with that of a standard nitrite solution.

**Standard**—Dissolve 0.231 gram of pure silver nitrite in 75 cc. of water. Add 0.150 gram of sodium chloride in 25 cc. of water. Filter the precipitate of silver chloride. Each cc. of filtrate contains 0.69 mg. of nitrite, which corresponds to 0.1 mg. of calcium.

#### CALCIUM BY THE TURBIDITY AS OXALATE

The nephelometric estimation of calcium may be made with calcium precipitated as the oxalate.<sup>38,39</sup> Only about 10 minutes is required for a determination.

<sup>35</sup> A. Astrug and M. Mousseron, *Compt. rend.* 190, 1558-9 (1930).

<sup>37</sup> See pp. 433-5.

<sup>38</sup> O. Schreiner and G. H. Failyer, *Dept. Agr. Bur. Soils Bull.* 31, 53 (1906).

<sup>39</sup> J. S. Sharpe, *Edinburgh Med. J.* 33, 27 (1926).

The method has been reported as unsatisfactory<sup>40</sup> due to the large size of individual particles. The effect of ammonium salts, excess of precipitant and the order of mixing the reagents is important. Standards cannot be kept for any length of time. The use of stabilizers is important.<sup>40a</sup> Experimental error has been estimated as possibly 20 per cent.

**Sample—Blood.** Place 2 cc. of blood in a tube and dilute to about 8 cc. Mix, add 1 gram of trichloroacetic acid and shake. Dilute to 10 cc., mix, and filter into a tube marked at 5 cc. Collect exactly 5 cc. of filtrate. Make distinctly alkaline with 1:1 ammonium hydroxide, then acidify with 30 per cent acetic acid until distinctly acid.

**Urine.** Follow the same treatment but add 0.5 gram of trichloroacetic acid instead of 1 gram. Add sufficient yellow color, such as diphenylamine orange, to give the same color to the standard as shown by the sample.

**Procedure—Balancing.** Add a few crystals of ammonium oxalate. Heat to boiling for a few minutes, cool slightly and add 1 cc. of pure glycerol. Dilute to 15 cc. with water, mix and compare in a nephelometer, with 2 cc. of standard treated in the same way.

**Duplication.** Add 10 cc. of saturated ammonium oxalate solution to each of 2 cylinders. To each add 1 cc. of concentrated ammonium hydroxide and 1 cc. of 0.1 per cent gelatine solution and mix well. Add the sample or an aliquot to 1 cylinder, dilute to 25 cc. and mix. Adjust the other cylinder by addition of a standard calcium solution until volume and reflection are matched.

**Standard—**Dissolve 0.1786 gram of pure calcium carbonate in 20 cc. of 1:1 hydrochloric acid. Make slightly alkaline with 1:3 ammonium hydroxide, then add a slight excess of 30 per cent acetic acid and dilute to 1 liter. Each cc. corresponds to 0.1 mg. of calcium oxide. This standard solution keeps indefinitely.

<sup>40</sup> A. I. Polinkavskii, *Trans. State Inst. for Testing Building Materials and Glass* (Moscow) 27, 11-26 (1929).

<sup>40a</sup> E. P. Garmash, *Zavodskaya Lab.* 1933, No. 8, 13-14.

## TURBIDIMETRIC APPROXIMATION OF CALCIUM AS THE OXALATE

Calcium may also be roughly estimated in water by comparison with standards.<sup>41</sup>

**Procedure**—To 10 cc. of sample add 1 cc. of 50 per cent acetic acid and mix. Add 1 cc. of 20 per cent potassium oxalate solution and mix. Compare after 10 minutes with a series of standards prepared at the same time.

## CALCIUM BY ALIZARIN

The tinctorial value of calcium alizarinate may be used for estimation of calcium.<sup>42</sup> It must first be separated from magnesium as they form similar salts with the dyestuff. Estimation of 0.1 mg. of calcium is accurate to 2 to 5 per cent.

**Sample**—Separate the calcium as the oxalate according to preceding methods,<sup>43</sup> centrifuge and wash as usual.

**Procedure**—To the calcium oxalate add 0.5 cc. of *N* hydrochloric acid and transfer to a test tube with water. Dilute to about 8 cc. and add 1 cc. of a 0.1 per cent alcoholic solution of alizarin for each 0.1 mg. of calcium expected. Warm to about 80° and add 5 drops of concentrated ammonium hydroxide. Mix well. A purple color results.

If available add a minute fragment of a crystal of calcium alizarinate to seed the solution for precipitation. Keep warm for 1 hour and let stand over night. Calcium is precipitated as the alizarinate in blue-black crystals. Filter on a Gooch crucible and wash the tube and filter well with 1:10 ammonium hydroxide. Filter paper should not be used. Do not suck air through the crucible as the carbon dioxide of the air will decompose the compound to some extent.

Place the tube in which the crystals were formed under the funnel and add 1 cc. of a 10 per cent solution of oxalic acid in 50 per cent alcohol. The crystals are decomposed and an orange solution obtained. Wash with 5 cc. of 95 per cent alcohol. The asbestos should at most be pink or a faint purple. Transfer this colored solution to a 50 cc. flask, rinse out the test tube with 1:10 ammonium hydroxide and adjust to

<sup>41</sup> W. D. Collins and Margaret D. Foster, *Ind. and Eng. Chem.* 15, 1078-80 (1923).

<sup>42</sup> P. P. Laidlaw and W. W. Payne, *Biochem. J.* 16, 494-8 (1922).

<sup>43</sup> See pp. 449-50,

neutrality by addition of 1:10 ammonium hydroxide. Dilute to volume and compare with the standard.

**Standard**—Dissolve 0.6 gram of alizarin in alcohol and dilute to 1 liter. Each cc. contains 6 mg. of alizarin which is equivalent to 1 mg. of calcium. Put 1 cc. of this solution in a 50 cc. volumetric flask. Add 1 cc. of 10 per cent oxalic acid solution in 50 per cent alcohol. Add 5 cc. of 95 per cent alcohol and render just alkaline with ammonium hydroxide. Dilute to 50 cc. The ammonium oxalate takes care of small differences in ammonia concentration by buffer action. A large excess gives a purple tint instead of red.

#### CALCIUM AS THE PICROLONATE

Calcium is precipitated with picrolonic acid and determined indirectly in terms of the picrolonic acid. When a dilute picrolonic acid solution is made turbid by reaction with bromine, excess bromine removed, and the solution made alkaline, the turbidity disappears and a red color is developed. The color is proportional to the concentration of picrolonic acid.<sup>44</sup> The red color varies somewhat between red and yellowish red. It is stable in the dark for at least 48 hours.

Magnesium, potassium, sodium and ammonium ions do not interfere. Iron and aluminum should be separated by precipitation, or kept in solution in the final alkaline medium by reaction with thiosalicic acid.

**Sample**—The sample solution should be only slightly acid in reaction. Evaporate excess acid if it is strongly acid. Transfer the solution, which should be about 5 cc., to a 10 cc. volumetric flask. Neutralize to a methyl orange end-point with 2 *N* sodium hydroxide solution. Add a few drops of 10 per cent thiosalicic acid solution, dilute to 10 cc. and filter.

Prepare the reagent by dissolving 2.64 grams of pure picrolonic acid in 1 liter of water by heating it on the water bath. Let cool over night and filter. The solution is about 0.01 *N*.

Pipet 1 or 2 cc. of filtrate into a tube. Add 3 times the volume of ice-cold picrolonic acid reagent. Let the calcium picrolonate stand in ice-water for 4 hours, shaking it every hour. Filter off the solution. Wash the precipitate 3 times with anhydrous ether. Add hot water to dissolve the precipitate and transfer the calcium picrolonate solution

<sup>44</sup> F. Alten, H. Weiland and E. Knippenberg, *Biochem. Z.* 265, 85-9 (1933).

to a 50 cc. volumetric flask. Wash out the filter with hot water, adding the washings to the 50 cc. flask.

**Procedure**—To the sample solution and to a standard solution <sup>45</sup> of calcium which has been treated the same as the sample, add 1 cc. of saturated bromine-water. The amount of calcium should be between 0.02 and 0.15 mg. Warm on a water bath for 10 minutes and add to each 10 cc. of alcohol. Let cool a half hour and add 2 cc. of 2 *N* sodium hydroxide solution. Dilute each to 50 cc. and compare.

#### BARIUM AS THE SULFATE

Sulfate radical is customarily estimated turbidimetrically by addition of barium chloride. The reverse use of the method requires addition of 1 cc. of 1:10 sulfuric acid to the barium solution instead of barium chloride solution to a sulfate solution. For further details see the methods for determination of sulfates.<sup>46</sup>

---

<sup>45</sup> See p. 451.

<sup>46</sup> See p. 611 et seq.

## CHAPTER XLVIII

### MAGNESIUM

#### MAGNESIUM AS THE PHOSPHATE BY REDUCTION WITH HYDROQUINONE

MAGNESIUM may be estimated indirectly from the phosphorus present in magnesium ammonium phosphate.<sup>1,2,3</sup> In general all of the methods applicable to phosphates or to calcium phosphate should apply. The published methods are more limited. One of the most widely used methods for phosphate under these conditions is by hydroquinone reduction.<sup>4</sup> Other reducing agents, such as stannous chloride<sup>5</sup> or aminonaphtholsulfonic acid,<sup>5a</sup> applied in detail for the estimation of phosphorus, may also be used. The results tend to be low, but with care in precipitation and washing this error can be limited to 3 per cent.<sup>6</sup>

**Sample—General.** In general, use the solution of magnesium separated from calcium oxalate in the determination of calcium as the phosphate by reduction with hydroquinone.<sup>7</sup>

**Urine.<sup>8</sup> High albumin content.** Destroy the albumin by wet or dry ashing,<sup>8a</sup> dissolve and proceed as below.

**Urine. Albumin absent.** Separation of calcium may be eliminated provided it is precipitated as the oxalate before addition of phosphate. Transfer 2 cc. of clear acid urine to a Pyrex test tube. Add 1 drop of methyl red solution and 1:3 ammonium hydroxide drop by drop until the color changes to brown. Add 5 per cent acetic acid if too much ammonium hydroxide is used. Add 1 cc. of 4 per cent ammonium oxalate and rub

---

<sup>1</sup> A. P. Briggs, *J. Biol. Chem.* 52, 349-55 (1922); *Ibid.* 59, 255-64 (1924).

<sup>2</sup> W. Denis, *J. Biol. Chem.* 52, 411-5 (1922).

<sup>3</sup> M. C. Franklin, *3rd Rept. of Director Univ. Cambridge Inst. Animal Path.* 1932-3, 130-4.

<sup>4</sup> Richard D. Bell and Edward A. Doisy, *J. Biol. Chem.* 44, 55-67 (1920).

<sup>5</sup> Josef Tischer, *Mikrochemie* 12, 65-86 (1932).

<sup>5a</sup> I. J. Cunningham and S. W. Josland, *New Zealand J. Sci. Tech.* 16, 28-9 (1934).

<sup>6</sup> F. S. Hammett and E. T. Adams, *J. Biol. Chem.* 52, 211 (1921).

<sup>7</sup> See pp. 454-5.

<sup>8</sup> Carl Urbach, *Biochem. Z.* 239, 182-5 (1931); *Ibid.* 241, 22-5 (1931).

<sup>8a</sup> See pp. 496-9.



down the sides of the tube with a policeman to precipitate calcium oxalate. Let stand 2 hours. The precipitate need not be separated.

*Organic Samples.*<sup>8b</sup> When ashed with nitric and perchloric acids the resulting solution is suitable for estimation of calcium, phosphorus, potassium and magnesium.

**Procedure**—To the sample in a Pyrex test tube marked at 15 cc. add 1 cc. of a 2 per cent solution of monopotassium phosphate and 1 cc. of concentrated ammonium hydroxide. Rub the tube with a policeman to aid the precipitation of magnesium ammonium phosphate. Let stand for 4 hours. Centrifuge for 10 minutes at 1500 r.p.m. Decant the clear upper layer and wash with 20 cc. of a mixture of 200 cc. of 95 per cent alcohol and 50 cc. of concentrated ammonium hydroxide. In doing this break up the precipitate well with a rod tipped with a policeman. Centrifuge, decant and wash again. Filtration on paper usually gives high results due to materials in the paper which give the phosphate reaction.<sup>9</sup>

Dissolve the residue of magnesium ammonium phosphate in 10 cc. of 0.01 *N* hydrochloric acid. Add 1 cc. of a 5 per cent ammonium molybdate solution and 1 cc. of a reagent containing 30 grams of sodium bisulfite and 1 gram of hydroquinone in 200 cc. of phosphorus-free water. Dilute to 15 cc. and compare at the end of one-half hour with 2 cc. of a standard monopotassium phosphate solution, corresponding to 0.1 mg. of phosphorus per cc., similarly treated at the same time. For details of that solution see the corresponding calcium determination.<sup>10</sup> Each cc. of standard corresponds to 0.0784 mg. of magnesium.

#### MAGNESIUM AS THE PHOSPHATE BY FERRIC THIOCYANATE

Magnesium may be determined in the same way as calcium by the reduction in color of ferric thiocyanate.<sup>11,12</sup> It is accurate to about 5 per cent.

**Sample**—Use that solution separated according to the procedure under calcium as the oxalate with ferric thiocyanate.<sup>13</sup> Add 0.5 cc. of concentrated sulfuric acid and evaporate to dryness. Ash. If small

<sup>8b</sup> For details of the method see pp. 498-9.

<sup>9</sup> F. S. Hammett and E. T. Adams, *J. Biol. Chem.* 54, 565 (1922).

<sup>10</sup> See pp. 454-5.

<sup>11</sup> W. M. Marriott and J. Howland, *J. Biol. Chem.* 32, 233-9 (1917).

<sup>12</sup> B. Kramer and F. F. Tisdall, *J. Biol. Chem.* 48, 223-32 (1921).

<sup>13</sup> See pp. 458-9.

amounts of black residue remain add a drop of concentrated sulfuric acid and repeat the ashing procedure.

Add to the ash 0.5 cc. of concentrated hydrochloric acid and 5 cc. of water. Warm and transfer to a 25 cc. beaker, using hot water for the transfer. Evaporate to about 3 cc. and add a drop of 0.01 per cent phenolsulfonephthalein solution. Add 1 cc. of 10 per cent ammonium phosphate solution and 1 cc. of concentrated ammonium hydroxide. Let stand over night to precipitate.

Transfer the contents of the beaker to a 15 cc. tube and centrifuge. Decant and wash with 10 cc. of 1:10 ammonium hydroxide, stirring thoroughly. Centrifuge and wash 4 more times with 5 cc. of 1:10 ammonium hydroxide. Wash once with 95 per cent alcohol made just alkaline with ammonium hydroxide. Decant and dry at 60°. Dissolve the residue in 5 cc. of 0.01 *N* hydrochloric acid. If necessary aliquot at this point.

**Procedure**—To the sample or an aliquot add exactly 2 cc. of ferric thiocyanate solution. This solution contains 5 cc. of 0.3 per cent ammonium thiocyanate solution, 5 cc. of 0.3 per cent ferric chloride solution and sufficient 1:1 hydrochloric acid to clarify the solution, diluted with water to make 50 cc. It should be at least one-half hour old before use. Dilute the sample to 10 cc. with 0.2 *N* hydrochloric acid and compare lengthwise with a series of standards prepared in the same way. The color is inversely, rather than directly, proportional to the magnesium content.

**Standard**—As a standard solution dissolve 0.102 gram of air dried magnesium ammonium phosphate in 100 cc. of 0.1 *N* hydrochloric acid and dilute to 1 liter with water. Each cc. contains 0.01 mg. of magnesium.

#### MAGNESIUM BY CONVERSION OF THE PHOSPHATE TO THE PHOSPHOMOLYBDATE

This indirect method<sup>14</sup> is of more historical than practical interest since more convenient methods are available. It is therefore given only in a brief form. The procedure is adequate for amounts up to 0.3 mg. of magnesium. Contamination of the reagents or sample with silica, phosphates or calcium must be avoided. The accuracy is about 5 per cent.

<sup>14</sup> Oswald Schreiner and William S. Ferris, *J. Am. Chem. Soc.* 26, 961-7 (1904).

**Procedure**—Prepare a phosphate reagent by dissolving 17.4 grams of dipotassium phosphate and 100 grams of ammonium chloride in 900 cc. of water. Add 50 cc. of concentrated ammonium hydroxide and dilute to 1 liter. To the sample, usually 50 cc., add 1 drop of concentrated ammonium hydroxide and 2 to 3 drops of a saturated solution of ammonium oxalate. Evaporate to dryness on the water bath. Add 1 cc. of the phosphate solution to this residue, stir with a glass rod and allow to stand for 2 hours. Wash the precipitate on a small filter with 5 cc. of 1:3 ammonium hydroxide. Wash with further quantities of 1:3 ammonium hydroxide, 5 cc. at a time, until a total of 50 cc. has been used. Wash the original dish with each portion before it is added to the filter. Wash the dish with 5 cc. of water and also wash the filter with pure water. Discard all of these washings.

Place a clean beaker under the filter and wash out the dish with 5 cc. of 1:2 nitric acid. Pour this same acid over the filter so as to dissolve all of the precipitate present. Wash the dish and filter with hot water, 5 cc. at a time, until nearly 45 cc. have been used. Cool, add 4 cc. of 5 per cent ammonium molybdate solution and dilute to 50 cc. The yellow color now appears and is at its greatest intensity after 20 minutes.

**Standard**—Prepare a standard by dissolving 0.5043 gram of pure disodium phosphate in water, adding 100 cc. of 1:2 nitric acid and diluting to 1 liter. This standard contains 0.1 mg. of phosphorus pentoxide per cc. which is equivalent to 0.0342 mg. of magnesium per cc. For the balancing or dilution method add to 5 cc. of the above solution, 70 cc. of water, 9 cc. of 1:2 nitric acid and 8 cc. of 5 per cent ammonium molybdate solution and dilute to 100 cc. After standing 20 minutes this solution attains the color representing 0.005 mg. of phosphorus pentoxide per cc. and is equivalent to 0.00171 mg. of magnesium per cc.

#### MAGNESIUM BY THE PHENOLIC PROPERTIES OF THE 8-HYDROXYQUINOLINE COMPLEX

When calcium has been previously precipitated, the phenolic properties of the 8-hydroxyquinoline complex with magnesium may be used for estimation of magnesium.<sup>15</sup> The results are accurate within 2 to 3 per cent. The method is satisfactory for blood with the calcium content

<sup>15</sup> Shun-ichi Yashimatsu, *Tôhoku J. Exptl. Med.* 14, 29-35 (1930); *Ibid.* 22, 463-6 (1934).

40 times as great as that of magnesium.<sup>16</sup> Sufficient reagent to allow for precipitation of the calcium must be present.

On standing, the hydroxyquinoline reagent loses its property of giving the magnesium complex and of reacting with Folin's phenol reagent.<sup>17</sup> It must therefore be freshly prepared. Reagents must be checked as not giving a starch-iodide reaction.

**Sample**—*Blood or Serum*. Transfer 1 cc. of oxalated blood or serum to a 15 cc. centrifuge tube. Mix with 7 cc. of water to lake. Add 1 cc. of 10 per cent sodium tungstate solution and mix. Add 1 cc. of 0.67 *N* sulfuric acid slowly with stirring. Stir vigorously and centrifuge after 10 minutes. If it is thought that any protein remains, heat in a boiling water bath, protected from evaporation, for 5 minutes. Pipet 5.0 cc. of the supernatant liquid into a 10 cc. centrifuge tube. Add 0.5 gram of ammonium chloride and mix. Place in a water bath at 80° and gradually raise to the boiling point. Add 7 drops of 1:2 ammonium hydroxide and mix. Add 2 drops of a 2 per cent solution of 8-hydroxyquinoline in 95 per cent alcohol. Stir vigorously until turbidity appears. Add 0.4 cc. of concentrated ammonium hydroxide, mix and heat for 10 minutes in boiling water. Decant and wash 3 times with 2 cc. of a hot 5 per cent solution of ammonium acetate to which sufficient concentrated ammonium hydroxide has been added to render it alkaline to phenolphthalein. Decant and dissolve the precipitate in 1 cc. of 0.5 *N* hydrochloric acid for use as sample.

*Urine*. Transfer 2 cc. of water to a centrifuge tube. Add about 0.5 gram of ammonium chloride and 0.5 cc. of urine. Heat in a water bath at 80° and gradually heat to boiling. Add 0.5 cc. of 1:2 ammonium hydroxide and mix. Add 0.5–0.7 cc. of a 5 per cent solution of 8-hydroxyquinoline in alcohol. Stir vigorously until turbidity appears. Heat in boiling water for 10 minutes, replacing the ammonia lost by evaporation. While hot centrifuge and wash the precipitate 3 times with 5 per cent ammonium acetate rendered slightly ammoniacal. Dissolve the precipitate in 1.5 cc. of *N* hydrochloric acid for use as sample.

**Procedure**—Transfer the solution to a 25 cc. volumetric flask with a few cc. of water. In a similar flask put 5 cc. of standard magnesium solution. Add 5 cc. of 20 per cent sodium carbonate solution followed

<sup>16</sup> R. Berg, W. Wolker and E. Skopp, *Mikrochem. Emich Festschr.* 1930, 18-22.

<sup>17</sup> F. Eichholtz and R. Berg, *Biochem. Z.* 225, 352-7 (1930).

by 1 cc. of phenol reagent.<sup>18</sup> Immerse the flasks in a boiling water bath for 30 seconds, cool to room temperature and dilute to the mark. Mix and compare.

**Standard**—Dissolve 10 grams of ammonium chloride in 100 cc. of 1 per cent magnesium sulfate solution. Add concentrated ammonium hydroxide until the solution is just alkaline to phenolphthalein. Heat gradually to boiling and add 10 cc. of 2 per cent solution of 8-hydroxyquinoline in 95 per cent alcohol. Boil for 10 minutes and filter on a Gooch crucible. Wash the precipitate with hot 1:40 ammonium hydroxide until the filtrate is colorless. Dry at 100°.

As a standard dissolve 0.1433 gram of the magnesium-quinoline complex in 20 cc. of 0.5 *N* hydrochloric acid. Dilute to 500 cc. Each cc. contains 0.01 mg. of magnesium. A similar standard prepared from 0.2150 gram of the complex contains 0.015 mg. of magnesium per cc.

#### MAGNESIUM BY 8-HYDROXYQUINOLINE BY DEVELOPMENT OF COLOR WITH IRON

The color of the 8-hydroxyquinoline complex with magnesium may also be developed with iron.<sup>18a</sup> This gives a green color. The method is particularly indirect in that it depends on the iron complex of 8-hydroxyquinoline which is derived from the corresponding magnesium complex.

**Procedure**—Transfer the sample solution in which the magnesium complex has been separated by 8-hydroxyquinoline<sup>18b</sup> and dissolved in acid, to a centrifuge tube. To another tube transfer 5 cc. of standard magnesium solution containing the magnesium as the 8-hydroxyquinoline complex.<sup>18c</sup> To each add 1 cc. of 10 per cent ammonium acetate solution and 0.5 cc. of 0.1 per cent ferric chloride solution. Mix well and add 3 drops of methyl red indicator solution. Add 0.25 *N* sodium hydroxide solution drop by drop until the color of the indicator changes. Centrifuge to separate the iron hydroxyquinolate. Wash each precipitate with water to remove excess iron.

Dissolve the precipitates in 95 per cent alcohol to which a drop of concentrated ammonium hydroxide has been added. Transfer to volu-

<sup>18</sup> See p. 183.

<sup>18a</sup> J. Lavollay, *Bull. soc. chim. biol.* 17, 432-8 (1935).

<sup>18b</sup> See p. 472.

<sup>18c</sup> See above.

metric flasks and dilute to a suitable volume for comparison by dilution or balancing.

#### MAGNESIUM BY DECOLORIZATION OF 8-HYDROXYQUINOLINE SOLUTION

In alkaline solution magnesium reacts quantitatively with 8-hydroxyquinoline to give a light green precipitate.<sup>19</sup> Calcium also reacts and must first be precipitated as the oxalate. No other materials naturally present in water interfere. For amounts from 0.5 mg. up, by taking proper aliquots, the colorimetric method is suitable. The original solution is bright yellow. The method is colorimetric by estimation of the color removed by precipitation as the magnesium salt. Results are accurate to about 10 per cent.

**Procedure**—The sample should be not greater than 50 cc. Add 20 cc. of 1:1 ammonium hydroxide and 50 cc. of a solution containing 0.5 gram of 8-hydroxyquinoline dissolved in 100 cc. of 95 per cent alcohol and diluted to 1 liter with 95 per cent alcohol. Filter and wash on the filter with 20 cc. of 1:10 ammonium hydroxide. Dilute the filtrate to 100 cc. and compare with the hydroxyquinoline solution diluted with an equal volume of water as standard. The loss represents the magnesium present. Each cc. of the original hydroxyquinoline solution is equivalent to 0.0416 mg. of magnesium.

#### MAGNESIUM BY 8-HYDROXYQUINOLINE BY CONVERSION TO A DYE

Various colorimetric methods are known in which magnesium is combined with 8-hydroxyquinoline. It can also be converted to a dye by coupling<sup>20</sup> in a manner similar to that for aluminum.<sup>21,22</sup> Amounts from 0.01 to 0.5 mg. of magnesium should be present in the sample used. The coupling agent is diazobenzene sulfonic acid.

Iron, aluminum, manganese, copper, zinc and titanium precipitate with the reagent and must therefore be removed. Calcium and barium are removed with ammonium oxalate. Aluminum is not precipitated by the reagent in the presence of sodium hydroxide and sodium tartrate. The positive error of the method is 2 to 6 per cent, when iron, aluminum, calcium and phosphate are removed.

<sup>19</sup> W. A. Hough and J. B. Ficklen, *J. Am. Chem. Soc.* **52**, 4752-5 (1930).

<sup>20</sup> F. Alten, H. Weiland and B. Kurmies, *Angew. Chem.* **46**, 697-8 (1933).

<sup>21</sup> F. Alten, H. Weiland and H. Loofmann, *Angew. Chem.* **46**, 668-9 (1933).

<sup>22</sup> See pp. 267-9.

**Reagent**—Add 4 grams of hydroxyquinoline to 8 cc. of glacial acetic acid. Stir and pour into 200 cc. of boiling water. Stir until dissolved and let cool before using.

**Procedure**—Add 0.5 cc. of saturated sodium acetate solution to 1 cc. of faintly acid sample. This sample should be free from ammonium-ion and acidified with acetic acid. Add 0.5 cc. of the reagent. Let stand for 3 hours at room temperature to precipitate iron and aluminum. Add 4 drops of saturated calcium oxalate solution to precipitate calcium and warm for 30 minutes on a water bath.

Transfer the clear upper layer, after centrifuging if necessary, to a clean tube. Add 0.5 cc. of cold water and wash the precipitate. Repeat the washing, adding the wash waters to the main solution.

Add 1 cc. of saturated sodium tartrate solution to keep any unprecipitated aluminum in solution, and add 0.3 cc. of reagent. Make alkaline with 1 cc. of 2 *N* sodium hydroxide solution. Let stand overnight to precipitate. Heat for 30 minutes on a water bath. Magnesium is now precipitated as the hydroxyquinoline compound. Centrifuge if necessary, and remove the clear upper layer. Wash the precipitate with 2 cc. of hot 1:10 ammonium hydroxide. Repeat the washing.

Dissolve the washed precipitate in 2 cc. of hot *N* hydrochloric acid. Transfer the solution to a 50 cc. flask with 3 cc. of *N* hydrochloric acid and dilute with water to about 30 cc. Add a mixture of 0.5 cc. of a solution of 8.6 grams of sulfanilic acid in 1 liter of 30 per cent acetic acid and 0.5 cc. of a solution of 2.85 grams of sodium nitrite per liter of water. Mix well, let stand for 10 minutes and make alkaline with 10 cc. of 2 *N* sodium hydroxide solution. Dilute to volume and mix.

Compare with a standard similarly prepared from 1 cc. of a standard magnesium solution.<sup>22a</sup> Direct preparation of the standard from an equivalent amount of hydroxyquinoline is not permissible.

#### MAGNESIUM BY AMMONIUM FERROCYANIDE

The procedure for determination of the total calcium and magnesium by the ferrocyanide method<sup>23,24</sup> has been given under calcium.<sup>25</sup> The method for determination of calcium in the presence of magnesium as the

<sup>22a</sup> See p. 473.

<sup>23</sup> F. Feigl and F. Pavelka, *Mikrochemie* 2, 85-91 (1924).

<sup>24</sup> Leonia Kriss, *Biochem. Z.* 162, 359-65 (1925).

<sup>25</sup> See pp. 453-4.

ricinoleate has also been given.<sup>26</sup> By difference from these 2 determinations the amount of magnesium may be estimated.

#### MAGNESIUM BY TITAN YELLOW

Titan Yellow, a derivative of dehydrothioparatoluidine sulfonic acid, is adsorbed from solution by magnesium hydroxide.<sup>27-29</sup> In a limited range the action may be used for estimation of magnesium. It gives an orange colored solution with 1 mg. of magnesium per liter, a red color with 5 mg. per liter, and a red flocculent precipitate with a greater concentration of magnesium. Aluminum and tin must be removed. Zinc if present, is first precipitated with ammonium sulfide. The precipitate need not be removed for a rough estimation. Precipitation of aluminum with ammonium hydroxide entrains most of the magnesium. A large concentration of ammonium salts interferes. Calcium deepens the red color. The dye in alkaline solution is itself a yellowish brown.

The method is simpler and more rapid than the usual methods. It may be applied to the solution from precipitation as the oxalate.<sup>30</sup> It is applicable to samples of 0.1 cc., and estimates quantities up to 0.015 mg. with an accuracy of 0.0001 mg. Use a piece of yellow-green, or grass-green glass as a light filter for greater accuracy. Protein must be absent.

**Sample—Urine.**<sup>29</sup> Dilute 0.1 or 0.2 cc. to 2 cc. Neither the natural color nor phosphates present interfere.

**Blood, Meat Extract, Cream.** Add 0.8 cc. of 10 per cent trichloroacetic acid to 0.2 cc. of sample, and centrifuge.<sup>32</sup> Pipet 0.5 cc. of clear supernatant liquid as the sample, neutralize with 10 per cent sodium hydroxide solution and dilute to 2 cc.

**Blood, Serum, Cream.**<sup>32</sup> Ash a 0.1 gram sample in a platinum dish below dull redness. Dissolve the ash in 1 drop of 5 per cent sulfuric acid and dilute to 2 cc.

**Meat.**<sup>32</sup> Ash 0.025 gram and follow the preceding method.

**Soil Extracts. Total Magnesium.**<sup>35</sup> Take an aliquot of the hydro-

<sup>26</sup> See pp. 451-2.

<sup>27</sup> I. M. Kolthoff, *Biochem. Z.* 128, 344 (1922); *Chem. Weekblad* 24, 254 (1927).

<sup>28</sup> H. D. Barnes, *J. South African Chem. Inst.* 11, 67-8 (1918).

<sup>29</sup> Jan Bečka, *Biochem. Z.* 233, 118-28 (1931).

<sup>30</sup> Arthur D. Hirschfelder, Earl R. Serles and Victor G. Haury, *J. Biol. Chem.* 104, 635-45 (1934).

<sup>32</sup> George P. Sanders, *J. Biol. Chem.* 90, 747-56 (1931).

<sup>35</sup> J. L. Steenkamp, *J. South African Chem. Inst.* 13, 64-70 (1930).



chloric acid extract. Neutralize with 4 *N* sodium hydroxide solution until a slight precipitate appears. Carefully add 1:9 hydrochloric acid drop by drop until this precipitate disappears. Precipitate iron and aluminum with a slight excess of 1:1 ammonium hydroxide. If there is considerable precipitate redissolve and reprecipitate. Combine the filtrates and washings, dilute to a known volume and take an aliquot equivalent to about 0.005 mg. of magnesium.

*Soil Extracts. Available Magnesium.* Evaporate an aliquot of the citric acid extract of the soil to dryness and ignite. Dissolve the ash in the minimum amount of 1:5 hydrochloric acid and proceed as for total magnesium in soil extracts.

**Procedure**—Measure volumes of standard magnesium solution of 0.0 to 1.2 cc. at 0.1 cc. intervals into a series of tubes. Dilute each to 2 cc. To each standard and to the sample add 0.2 cc. of a 5 per cent dialyzed agar solution prepared by heating to 40°, 0.1 cc. of a 0.2 per cent solution of Titan Yellow in 50 per cent alcohol and, after mixing thoroughly, 0.5 cc. of 2 *N* sodium hydroxide solution. Compare. The solutions are stable for 2 days when not exposed to light.

**Standard**—Dissolve 0.1014 gram of hydrated magnesium sulfate in water and dilute to 1 liter. Each cc. is equivalent to 0.01 mg. of magnesium.

#### MAGNESIUM BY CURCUMIN

Curcumin forms a yellow to orange lake with magnesium<sup>36</sup> which is suitable for colorimetric estimation.<sup>37,38</sup> Phosphates affect the color and suggest that the product is a magnesium phosphate-curcumin lake. Iron should be removed as its color will interfere. Borates up to 8 mg. do not alter the color. The suspension is desirably stabilized by a solution such as that of starch glycerite.<sup>39</sup>

**Sample**—Use a solution containing about 2 cc. of concentrated hydrochloric or nitric acid per liter. Calcium need not be absent and phosphates should be present.

<sup>36</sup> I. M. Kolthoff, *J. Am. Chem. Soc.* 50, 395 (1928).

<sup>37</sup> W. E. Thrun, *Ind. Eng. Chem., Anal. Ed.* 4, 426-7 (1932).

<sup>38</sup> F. Thompson, *Ind. Chemist* 10, 142 (1934).

<sup>39</sup> W. E. Thrun, *Ind. Eng. Chem., Anal. Ed.* 2, 8 (1930).

**Procedure**—Dilute an aliquot in a 50 cc. tube or volumetric flask to about 40 cc. Add 2 cc. of a filtered aqueous solution of starch glycerite. Mix well and add accurately 4 drops of 1 per cent alcoholic solution of curcumin. Mix and add 5 cc. of 4 *N* sodium hydroxide solution. Mix well, dilute to volume and mix. Compare with standards prepared at the same time in the same way from a standard magnesium solution containing phosphates.

**Standard**—Dissolve 0.203 gram of hydrated magnesium sulfate and about 0.2 gram of tricalcium phosphate in water containing 2 cc. of concentrated nitric acid. Dilute to 1 liter. Each cc. contains 0.02 mg. of magnesium.

#### MAGNESIUM AS THE ALIZARINATE

Although not described, there seems to be no reason why magnesium cannot be determined by the method described for calcium.<sup>40,41</sup>

The magnesium separated by the methods previously given should be precipitated and treated as described for calcium. The calcium standard is equivalent to 0.608 mg. of magnesium per cc.

#### MAGNESIUM AS THE OLEATE

This procedure given for calcium may also be applied to magnesium.<sup>42</sup>

**Sample**—Apply the usual methods previously described<sup>43</sup> in detail for separation of magnesium from calcium.

**Procedure**—Dilute the sample in a 50 cc. volumetric flask to about 45 cc. In a similar flask take 1 to 5 cc. of a solution containing 0.02 mg. of magnesium per cc.<sup>44</sup> To each add 2 cc. of a solution containing 100 grams of ammonium chloride and 10 cc. of concentrated ammonium hydroxide per liter. Mix and add 1 cc. of a solution of 2 grams of oleic acid and 0.5 gram of potassium hydroxide per liter of 60 per cent alcohol. Dilute each to 50 cc., mix and let stand for 2 hours. Compare the pale yellow color nephelometrically.

<sup>40</sup> P. P. Laidlaw and W. W. Payne, *Biochem. J.* 16, 494-8 (1922).

<sup>41</sup> See pp. 465-6.

<sup>42</sup> A. Gregoire and T. Sola, *Bull. soc. chim. Belg.* 32, 131 (1923).

<sup>43</sup> See pp. 458-9, 468-9.

: above.

## MAGNESIUM NEPHELOMETRICALLY AS THE PHOSPHATE

Magnesium may be estimated nephelometrically as the phosphate.<sup>45</sup> Interfering elements are removed by precipitation with ferrocyanide, oxalate, etc. The method is accurate to within 12 per cent and can be carried out in 15–20 minutes.

**Procedure**—To each of 2 cylinders add 10 cc. of 0.2 *N* trisodium phosphate solution and 10 cc. of 1:9 ammonium hydroxide and dilute to 50 cc. To 1 cylinder add 10 cc. of sample drop by drop with stirring. Dilute to 80 cc. To the other cylinder add standard magnesium solution <sup>46</sup> until the solutions match, either nephelometrically or turbidimetrically.

<sup>45</sup> E. V. Vasil'eva, *Zavodskaya Lab.* 1933, No. 8, 10-13.

<sup>46</sup> See p. 477.

## CHAPTER XLIX

### PHOSPHORUS

#### PHOSPHORUS AS PHOSPHOMOLYBDATE

ALTHOUGH the gravimetric method of analysis for phosphorus by precipitation of the phosphomolybdate is very inaccurate for small amounts of phosphorus, the solutions of these small amounts of phosphomolybdate may be determined titrimetrically or compared colorimetrically with a very satisfactory degree of accuracy.<sup>1-9</sup>

The solution contains  $\text{H}_3\text{PO}_4 \cdot 12\text{MoO}_3$ . In the presence of silica, germanium or arsenic the corresponding compounds,  $\text{H}_4\text{SiO}_4 \cdot 12\text{MoO}_3$ ,  $\text{H}_4\text{GeO}_4 \cdot 12\text{MoO}_3$  and  $\text{H}_3\text{AsO}_4 \cdot 12\text{MoO}_3$  will be present.<sup>10</sup> The color of the phosphate complex is markedly affected by temperature. A method has been developed for estimation of silica and phosphorus in the presence of each other.

Titanium gives a greenish yellow color with ammonium molybdate and nitric acid. It does not fade in an hour. Preliminary heating to  $100^\circ$  for two hours with nitric acid renders titanium completely insoluble. Vanadium in neutral solution gives a yellow color which is permanent for several hours. On adding nitric acid this fades completely within 5 minutes. In acid solution, therefore, no interference will occur from this source.

Ammonium salts must not exceed 0.05 per cent, as they reduce the color developed. Aluminum in amounts greater than 0.1 p.p.m. prevents satisfactory development of color. Iron must not exceed 20 p.p.m. This is several times the amount to be expected in a soil extract but if there

<sup>1</sup> J. West-Knights, *Analyst* 5, 195 (1880).

<sup>2</sup> Charles Lepierre, *Bull. soc. chim.* [3], 15, 1213 (1896).

<sup>3</sup> Adolf Jolles and F. Neurath, *Monatsh. Chem.* 19, 5 (1898).

<sup>4</sup> Adolf Jolles, *Arch. Hyg.* 34, 22 (1899).

<sup>5</sup> A. G. Woodman and L. L. Cayvan, *J. Am. Chem. Soc.* 23, 96 (1901).

<sup>6</sup> A. G. Woodman, *Ibid.* 24, 735 (1902).

<sup>7</sup> F. P. Veitch, *Ibid.* 25, 169 (1903).

<sup>8</sup> Oswald Schreiner, *Ibid.* 25, 1056 (1903); *Ibid.* 26, 808 (1904).

<sup>9</sup> A. T. Lincoln and Perry Barker, *Ibid.* 26, 975 (1904).

<sup>10</sup> Paul Krumholz, *Z. anorg. allgem. Chem.* 212, 91-6 (1933).

is any question, a rough estimation of iron may be made by the thiocyanate method. If the amount of organic matter in the solution is considerable it must be removed by ignition. There is an important loss of phosphorus unless basic radical is in excess over the amount of phosphate. The stability of the final suspension is somewhat increased by addition of 5 cc. of glycerol to sample and standards during dilution.<sup>11</sup> As small an amount of phosphate as 0.002 mg. of phosphorus pentoxide may be estimated by accurate dilution of the standard. By heating the standard and sample to 60° the delicacy may be further increased to 0.001 mg. of phosphorus pentoxide. No entirely satisfactory solution for permanent standards has been found. Picric acid is best but gives a greenish color and also gradually increases in color intensity due to solution of alkali from the glass. Standards which have been heated have less than the original color when cooled again. A series of standards must be replaced daily as the darker solutions precipitate out and the lighter solutions fade. The fact that the color is not complete for some minutes renders the duplication method inconvenient.

A series of determinations by this method on samples containing from 1-5 p.p.m. of phosphorus pentoxide varied by about 10 per cent from the theoretical values. The effect of several other salts has been studied and the maximum amounts permissible within the limit of error of the method are tabulated below.<sup>12</sup>

	p.p.m.	<i>N</i>
Sodium sulfate decahydrate	500	0.003
Potassium nitrate	1000	0.01
Magnesium sulfate (hydrate)	1000	0.008
Potassium hydrogen sulfate	1500	0.02
Calcium nitrate	2000	0.025
Magnesium nitrate	2000	0.03
Sodium chloride	2000	0.035
Potassium ethyl sulfate	3000	0.035
Sodium nitrate	5000	0.06

The relatively large tolerance of magnesium nitrate, a common oxidizing agent for organic matter, will be noted.

<sup>11</sup> Zola Y. Tsen, *Acad. Sinica Inst. Chem. Mem.* 4, 1-5 (1931).

<sup>12</sup> Clarence Estes, *J. Am. Chem. Soc.* 31, 247 (1909).

**Sample**—If organic matter is to be removed take 10 to 100 cc. of solution, which should contain less than 0.5 mg. of phosphorus, as a sample. Add 5 cc. of 1:5 nitric acid and 5 cc. of 1 per cent magnesium nitrate solution. Evaporate to dryness in a porcelain evaporating dish and ignite until all carbon is removed. The loss of phosphorus pentoxide under these conditions is approximately 0.06 p.p.m.

If the organic matter is equivalent to less than 0.3 p.p.m. of phosphorus pentoxide it may be estimated by comparison with the series of phosphorus standards and this blank subtracted from the total reading after development of color and comparison with standards. If the amount of color of the solution due to organic matter is negligible, filter to remove any suspended matter, discarding the first 100 cc. of filtrate.

**Procedure**—To 10–100 cc. of solution, which must contain less than 5 mg. of phosphorus, add 5 cc. of 1:5 nitric acid and 5 cc. of 1 per cent magnesium nitrate solution and evaporate to dryness on a water bath. Heat the residue at 100° for 2 hours. If the substance contains no titanium or silica this evaporation and dehydration may be omitted. Dissolve the residue after dehydration in 5 cc. of cold water. Repeat if much calcium and magnesium are present. It will not be necessary to filter as the loss due to filtration would be greater than that due to the presence of a small amount of precipitate of silica. Add 5 cc. of 1:5 nitric acid and 4 cc. of a 5 per cent solution of neutral ammonium molybdate. After 3 minutes the color is suitable for comparison with a standard or series of standards developed in the same way at the same time.

**Standard**—As a standard dissolve 0.5043 gram of pure crystallized disodium phosphate in water, add 100 cc. of 1:5 nitric acid and dilute to 1 liter. This solution contains 0.1 mg. of phosphorus pentoxide per cc. After some months the strength of this standard increases in a stoppered bottle due to solution of silica from the glass. The presence of acid delays deterioration from this source.

For a series of standards use 100 cc. Nessler tubes with portions of the above standard at 1 cc. intervals from 1 to 10. To each add 5 cc. of 1:5 nitric acid and 4 cc. of 5 per cent neutral ammonium molybdate solution and dilute to 50 cc. Above this concentration, the color becomes too intense to read satisfactorily. A lower series of 10 standards may be prepared varying from 0.1 to 1.0 cc. of the standard and if desired a further dilute series may be prepared by accurately diluting 10 cc. of the

standard to 100 cc. or 500 cc. and using 0.1–1.0 cc. of this dilute standard.

For use with the dilution or balancing methods dilute 10 cc. of the standard with 75 cc. of water and 9 cc. of 1:5 nitric acid. Add 8 cc. of the molybdate solution and dilute to 100 cc. This standard will contain 0.01 mg. of phosphorus pentoxide per cc. It is essential that the sample solution and the standard contain exactly the same amounts of acid and molybdate, due to the well known variability in the precipitation of ammonium molybdate under varying conditions.

#### PHOSPHORUS, SEPARATED BY PRECIPITATION AS MAGNESIUM AMMONIUM PHOSPHATE

This method is similar to that for magnesium.<sup>13</sup> The phosphate is precipitated as magnesium ammonium phosphate<sup>14</sup> by addition of an excess of magnesia mixture, the precipitate is filtered off, dissolved in nitric acid and developed colorimetrically by the addition of a solution of ammonium molybdate. The advantages of this method are that all silica is removed from the solution, thus preventing errors due to silicomolybdate, and that the organic matter is removed from the final solution. Satisfactory samples should range between 0.5 mg. and 4 mg. of phosphorus pentoxide. Results obtained in the presence of various disturbing factors were uniformly satisfactory.

**Procedure**—The sample is usually 50 cc. of solution. Add 1 drop of concentrated ammonium hydroxide and 2 to 3 drops of a saturated solution of ammonium oxalate to the sample and evaporate to dryness on the water bath. A magnesia mixture is prepared from 13 grams of crystallized magnesium chloride, 20 grams of ammonium chloride and 50 cc. of concentrated ammonium hydroxide in 900 cc. of water. This is diluted to one liter. Add 1 cc. of this solution to the dried precipitate and mix with a glass rod. After standing for two hours wash the precipitate on the filter with 5 cc. of 1:9 ammonium hydroxide. Wash the dish and filter with further successive portions until the volume of the filtrate reaches approximately 50 cc. Wash the dish and filter with 5 cc. of water and discard the washings. This eliminates a trace of silica present in ammonium hydroxide. Place a clean beaker to catch the solution of the precipitate. Add 5 cc. of 1:5 nitric acid to dissolve any precipitate remaining on the dish and carefully dissolve the precipitate

<sup>13</sup> O. Schreiner and W. S. Ferris, *J. Am. Chem. Soc.* 26, 961 (1904).

<sup>14</sup> O. Schreiner and B. E. Brown, *Ibid.* 26, 1463 (1904).

on the filter with this same acid. Wash the dish and filter with successive 5 cc. portions of hot water until the filtrate amounts to 45 cc. Add 4 cc. of a 5 per cent solution of neutral ammonium molybdate and compare after 20 minutes.

**Standard**—Prepare the standard by dissolving 0.5043 gram of disodium phosphate in water with the addition of 100 cc. of 1:5 nitric acid, and diluting to 1 liter. This standard solution contains 0.1 mg. of phosphorus pentoxide per cc. A series of standards may be prepared by using from 1 to 10 cc. of this solution. Dilute to 45 cc. and add 4 cc. of 5 per cent neutral ammonium molybdate solution. Dilute each to 50 cc. A duplicate cannot be prepared since the color is so long in developing. A standard for the dilution or balancing method is prepared by adding to 10 cc. of the above standard, 70 cc. of water, 9 cc. of 1:5 nitric acid and 8 cc. of 5 per cent neutral ammonium molybdate solution, and diluting to 100 cc. This standard, which contains 0.01 mg. of phosphorus pentoxide per cc. must stand for 20 minutes before use in order to bring out the color.

#### PHOSPHORUS BY MOLYBDENUM SULFIDE

Another closely related method is to dissolve the phosphorus, separated as phosphomolybdate, in sodium hydroxide solution and convert the accompanying molybdenum to the sulfide.<sup>15</sup> The method was originally designed for use with iron and steel samples and when properly applied will detect differences as small as 0.001 mg. of phosphorus.

**Sample**—*Minerals*. Separate the phosphomolybdate as for determination in that form.<sup>16</sup>

*Iron and Steel*. Follow the usual volumetric method through separation of phosphorus as phosphomolybdate.

**Procedure**—Filter the precipitated phosphomolybdate and wash well with 1:10 nitric acid. Place the funnel with the precipitate over a 100 cc. volumetric flask. Moisten the paper with hot water and add 0.1 *N* sodium hydroxide solution from a calibrated pipet or buret until the precipitate is dissolved. Add a measured excess which should be about half the amount required to dissolve the precipitate. Excess is essential

<sup>15</sup> T. E. Hewitt, *J. Am. Chem. Soc.* **27**, 121 (1905).

<sup>16</sup> See p. 482.



or a black color is obtained with hydrogen sulfide. Dilute to volume and mix.

Take a suitable aliquot of the sample solution according to the nature of the sample. Dilute to about 25 cc. in a Nessler tube. Pass hydrogen sulfide through for 5 minutes. Saturation is essential for development of the proper color. Place in a boiling water bath for 5 minutes. Remove, cool, dilute to 50 cc. and compare with a standard. The color after heating is stable for 2 hours.

**Standard**—Precipitate about 0.1 gram of ammonium phosphomolybdate, filter, wash with 1:10 nitric acid and dry at 120–130°. More than 0.4 gram cannot be dried satisfactorily. Dissolve 0.0614 gram of this precipitate following the procedure above for solution of the phosphomolybdate precipitate from the sample, and dilute to 1 liter. Each cc. of the standard is equivalent to 0.001 mg. of phosphorus.

#### PHOSPHORUS IN THE PRESENCE OF SILICA BY AMMONIUM MOLYBDATE

See "Silica and Phosphorus by Ammonium Molybdate," in the next chapter.

#### PHOSPHORUS AS THE PHOSPHOVANADIOMOLYBDATE

This reaction has been developed for estimation of phosphorus in iron and steel. It is accurate to about 0.005 per cent of phosphorus.<sup>17,18</sup>

**Sample**—Dissolve 1 gram of steel in 20 cc. of 1:1 nitric acid in a 100 cc. flask. To the hot solution add 10 cc. of an 0.8 per cent solution of potassium permanganate. Boil, let cool and dissolve the precipitated manganese dioxide by addition of a 6 per cent sulfur dioxide solution or of 3 per cent hydrogen peroxide solution.

**Procedure**—Prepare a solution containing 2.345 grams of ammonium vanadate dissolved in 200 cc. of hot water. Add 20 cc. of 1:1 nitric acid and dilute to 1 liter.

Add 10 cc. of this solution to the sample, boil off excess sulfur dioxide or hydrogen peroxide and add 10 cc. of a 10 per cent ammonium

<sup>17</sup> Robert Schroder, *Stahl u. Eisen*, 38, 816-7 (1918).

<sup>18</sup> G. Misson, *Chem.-Ztg.* 32, 633 (1922); *Ann. chim. anal. chim. appl.* 4, 267 (1922).

molybdate solution. A yellow solution of phosphovanadomolybdate is formed. After 10 minutes compare with the color of steel samples of known phosphorus content similarly treated. Standards should cover the range to be examined with intervals of about 0.01 per cent. They must be renewed weekly. A suitable amount of phosphorus for estimation is 0.03 to 0.10 per cent with a 1 gram sample.

#### PHOSPHORUS BY REDUCTION OF THE PHOSPHOMOLYBDATE

The methods are so numerous that they have for convenience been subdivided into three steps: First, preparation of sample which includes the amount, all steps up to ashing, and the amount of standard to be used for comparison; second, ashing, not necessary in all cases; third, methods of color development for the determination. The applications of the typical methods given are much more numerous than can be listed, to plankton<sup>18a</sup> for example.

**Sample—Inorganic Phosphorus in Urine.** A suitable sample should contain 0.2 to 0.5 mg. of phosphorus. This amount is usually present in 1-2 cc. If results cannot be obtained without removal of organic matter, as may be the case with the molybdic acid reagent, precipitate organic matter by the method for inorganic phosphorus in blood and use the filtrate. The standard should contain a corresponding amount, 0.2-0.5 mg. of phosphorus.

**Total Phosphorus in Urine.** Ash 1 cc. and determine by comparison with a standard containing from 0.1-0.5 mg. of phosphorus. The concentration of standard necessary may be estimated from results obtained on inorganic phosphorus.

**Organic Phosphorus in Urine.**<sup>19</sup> This cannot be accurately determined by difference because it is relatively small. Measure 20 cc. of urine into a 25 cc. volumetric flask. Render faintly alkaline with powdered barium hydroxide, thus precipitating the inorganic phosphorus, dilute to volume and filter. To remove excess barium measure 20 cc. of filtrate into a 25 cc. volumetric flask, render very faintly acid with sulfuric acid, dilute to volume and filter. Avoid the use of excess sulfuric acid. One cc. of this filtrate is equivalent to 0.64 cc. of the original urine.

Ash 10 cc. of the above filtrate and determine phosphorus by comparison with a standard equivalent to 0.025 mg. of phosphorus. If this

<sup>18a</sup> L. H. N. Cooper, *J. Marine Biol. Assoc.* 19, 755-9. (1934).

<sup>19</sup> Carl Urbach, *Biochem. Z.* 239, 182-5 (1931).

standard is not dark enough it is better to repeat the digestion with a smaller amount of the filtrate than to increase the strength of the standard.

Preliminary removal of inorganic phosphorus with magnesium citrate is probably more accurate but requires a much longer time. Organic phosphorus must be determined on fresh urine,<sup>20</sup> as the organic compound of phosphorus is partially or completely decomposed in the course of a few weeks, even in a few days if ammonia is present.

*Total Phosphorus in Whole Blood.* Pipet 2 cc. of blood into a 25 cc. calibrated flask half full of water, make to volume and mix. Ash 2 cc. of this diluted blood, develop and compare. The standard should contain 0.03 or 0.06 mg. of phosphorus.

*Total Phosphorus in Plasma and Serum.* Ash 0.5 cc. of plasma or serum, or dilute to one-quarter strength with distilled water and ash a 2 cc. sample. Develop and compare with 0.1 mg. of phosphorus.

*Total Phosphorus in Corpuscles.* Centrifuge blood for 10 minutes at 4000 r.p.m. and decant the plasma as quickly as possible. Wash once with a volume of 0.9 per cent salt solution equal to the plasma, by shaking and centrifuging. If done quickly there will be no significant loss of phosphorus by dialysis. Measure 1 cc. of corpuscles into a 25 cc. volumetric flask, rinse the pipet with water and add the rinsings to the flask. Dilute to volume, mix, pipet 2 cc. as sample and ash. Develop and compare with 0.03 or 0.06 mg. of phosphorus.

*Inorganic Phosphorus in Blood.* Measure 2 cc. and run into 8 cc. of 10 per cent trichloroacetic acid<sup>21</sup> solution in a small Erlenmeyer flask, rotating gently during the addition. Blood containing more than 3 mg. per cc. of potassium oxalate as anticoagulant cannot be used. Stopper with a clean, dry rubber stopper and shake vigorously. Filter through ashless paper, protecting from evaporation, and use a 5 cc. sample of the filtrate equivalent to 1 cc. of blood. Compare with a standard containing 0.02 mg. of phosphorus. Add 4 cc. of 10 per cent trichloroacetic acid to the standard to render conditions comparable.

If necessary to reach the desired sensitivity phosphate may be added to the blood and later subtracted from the result. By the use of this high concentration of trichloroacetic acid, filtration is rapid.<sup>22</sup> Laking the

<sup>20</sup> G. C. Mathison, *Biochem. J.* 4, 233 (1904).

<sup>21</sup> A blank test must be made on the trichloroacetic acid. If necessary it is re-distilled.

<sup>22</sup> A. Hiller and D. Van Slyke, *J. Biol. Chem.* 53, 257 (1922).

blood is unnecessary.<sup>23</sup> The trichloroacetic acid procedure above is to be used instead of Bloor's acid ammonium sulfate or Folin and Wu's phosphotungstic acid because the coagulating agent can be completely volatilized for determination of acid-soluble phosphorus.

If desired, the filtrate and standard may be neutralized with 40 per cent sodium hydroxide solution, giving suitable attention to volumes before and after.<sup>24</sup> In unlaked blood inorganic phosphorus in the plasma increased 20 per cent in 5 hours at 37° due to enzyme hydrolysis. The rate of increase is 10 per cent in 5 minutes after laking. No such effect is obtained in the presence of trichloroacetic acid.

*Inorganic Phosphorus in Plasma and Serum.* Follow the procedure for whole blood and compare with a 0.04 mg. phosphorus standard.

*Inorganic Phosphorus in Corpuscles.* Pipet 5 cc. into a 10 cc. volumetric flask and rinse the pipet with warm water, letting the washings run into the flask. Dilute to volume, mix well and let stand for 10 minutes with occasional shaking to allow the corpuscles to lake. Run 5 cc. slowly with mixing into 18 cc. of 10 per cent trichloroacetic acid solution in a 25 cc. volumetric flask. Mix well and dilute to volume with 10 per cent trichloroacetic acid solution. Filter a portion, develop the color in 5 cc. of filtrate and compare with a standard containing 0.05 mg. of phosphorus. Add 4 cc. of 10 per cent trichloroacetic acid to the standard to make conditions comparable.

*Acid Soluble Phosphorus in Blood, Plasma, Serum or Corpuscles.* Ash 5 cc. of fresh filtrate from the determination of inorganic phosphorus. Develop and compare with a standard containing 0.1 mg. of phosphorus. One cc. may be used in a 10 cc. test tube with suitable reduction of the amounts of reagents.

This determination must be carried out on fresh filtrate as hydrolysis of the organic phosphorus present begins at once and in 24 hours the acid-soluble phosphorus and the inorganic phosphorus are approximately identical.

*Lipoid Phosphorus in Blood, Plasma, Serum or Bile.*<sup>25-27</sup> To 75 cc. of a 3:1 mixture of anhydrous alcohol and redistilled ether in a 100 cc. volumetric flask add 5 cc. of oxalated whole blood, plasma, serum or bile in a fine stream, swirling during the addition. Immerse the flask in hot

<sup>23</sup> C. H. Fiske and Y. Subbarow, *Ibid.* 66, 375 (1925).

<sup>24</sup> H. D. Kay and R. Robison, *Biochem. J.* 18, 755 (1924).

<sup>25</sup> F. S. Randles and A. Knudson, *J. Biol. Chem.* 53, 53 (1922).

<sup>26</sup> E. J. Baumann, *Ibid.* 59, 667 (1924).

<sup>27</sup> J. C. Whitehorn, *Ibid.* 62, 136 (1924).

water and continue to rotate until the contents boil. Remove at once, cool in water and dilute to volume with the alcohol-ether mixture. Filter with precautions against evaporation.

Evaporate 10 cc. of the filtrate to dryness, ash and develop the color in comparison with a standard containing 0.05 mg. of phosphorus. The extraction may also be carried out by adding 1 cc. of blood to 40 cc. of the extraction mixture, diluting to 50 cc. and using 25 cc. of filtrate as sample.

The original Whitehorn method is stated to give differences of 10 to 15 per cent which are reduced to 1 per cent by more accurate methods of measurement and by the use of 6 cc. of a 20 per cent solution of anhydrous sodium sulfite in place of 4 cc.<sup>28</sup> for the development of color.

*Lipoid Phosphorus in Corpuscles.* To hemolyze, dilute with an equal volume of warm water and let stand for 10 minutes. Extract the lipoids from 6 cc. of this dilution as for whole blood except that the flask is shaken occasionally for 30 minutes to avoid the tendency of the precipitate to aggregate before heating. Use 10 cc. of extract, an aliquot corresponding to 0.3 cc. of corpuscles, and compare with a standard containing 0.01 mg. of phosphorus.

*Lipoid Phosphorus in Tissue, Seeds, and Similar Materials.*<sup>29</sup>—Mix 1.0–1.5 grams of finely ground tissue with 3 grams of plaster of Paris and dry in a vacuum desiccator over sulfuric acid. Pulverize the mass with a little well-washed pulverized glass in a mortar. Filter on a Gooch crucible or fat-free paper. Wash the mortar with ether and pour the washings over the main precipitate.

Extract with a Wiley extractor using anhydrous ether for 6 hours and absolute alcohol over night. Repeat the next day. Extract the third day with absolute alcohol. Concentrate to a few cc. and dry in a vacuum desiccator over concentrated sulfuric acid. Dissolve the fatty matter with anhydrous chloroform and filter several times until clear. Wash the filter with hot chloroform. If desired the extract may be evaporated and dried to give the weight of total extract and redissolved in chloroform. Dilute the solution to 25 cc., pipet out 2–5 cc. and evaporate to dryness. Ash by one of the usual methods and determine phosphorus in comparison with a standard chosen according to the nature of the sample.

As an alternative method,<sup>30</sup> dry seeds, or other sample, and grind to

<sup>28</sup> A. Karssen and C. R. van Wering, *Biochem. Z.* 253, 427-30 (1932).

<sup>29</sup> E. J. Baumann, *J. Biol. Chem.* 59, 672 (1924).

<sup>30</sup> N. B. Guerrant, *J. Am. Chem. Soc.* 48, 2185 (1926).

pass a 40 mesh sieve. Add 25 cc. of a mixture of 1 part of anhydrous ether with 4 parts of absolute alcohol to a 1 gram sample in a centrifuge tube. Stopper and shake for 10 minutes, preferably mechanically. Centrifuge to settle the insoluble matter. Pipet a 5 cc. sample of the clear layer, evaporate the solvent, ash and determine phosphorus on the residue. This method of extraction originally proposed by Schultz<sup>31</sup> extracts some inorganic phosphorus if 95 per cent alcohol and ordinary ether are used.

*Estimation of Lipoid Phosphorus.* The total phosphorus less the acid-soluble phosphorus gives a close approximation of the lipoid phosphorus present, often with an accuracy equal to that of the available methods of determination.

*Hydrolysable Organic Phosphorus in Blood.* Blood contains a hydrolysable organic phosphate which is largely precipitated in obtaining the plasma.<sup>32</sup> To 5 cc. of clear plasma add reagents as usual for the hydroquinone method, omitting the sulfite. Mix, stopper loosely and heat in boiling water for fifteen minutes. Cool, add sulfite, dilute to 10 cc., mix and read after standing the usual time.

Dilute 2 cc. of whole blood filtrate with 3 cc. of water and determine in the same way except that the period of heating is one hour. Compare each with a 0.03 mg. standard. The difference after allowing for the aliquots taken represents hydrolysable organic phosphorus.

*Animal Urine.*<sup>32a</sup> Digest 25–50 cc. with 5–10 cc. of concentrated sulfuric acid until copious fumes are no longer evolved. By addition of 10–15 cc. of concentrated nitric acid emission of offensive odors is avoided. Add 0.5 cc. or if necessary, 1.0 cc. of perchloric acid and proceed as usual in perchloric acid digestion.<sup>32b</sup>

*Milk.*<sup>33</sup> Add 2 cc. of milk to 5 cc. of water in a graduated cylinder. Add 2 cc. of 20 per cent trichloroacetic acid and dilute to 10 cc. Mix and filter to obtain a clear solution. Use 5 cc. of filtrate, equivalent to 1 cc. of milk, as sample.

*Mineral Phosphate in Wine.*<sup>34</sup> Use the original wine without destruction of organic matter following a suitable modification of the procedure for urine.

<sup>31</sup> E. Schultz, *Z. physiol. Chem.* 20, 225 (1895); *Chem.-Ztg.* 21, 374 (1897); *Ibid.* 28, 751 (1904).

<sup>32</sup> T. F. Zucker and M. Gutman, *Proc. Soc. Exp. Biol. Med.* 20, 133 (1922-3).

<sup>32a</sup> H. W. Gerritz, *Ind. Eng. Chem., Anal. Ed.* 7, 116-8 (1935).

<sup>32b</sup> See pp. 498-9.

<sup>33</sup> Antolin Pena, *Lait* 11, 942-5 (1931).

<sup>34</sup> G. Deniges, *Mikrochem. Pregl Festschr.* 1929, 27-45.

*Total Phosphate in Wine.* Ash a suitable sample.

*Argillaceous Minerals.*<sup>35</sup> Fuse the sample with potassium bisulfate or fuse with mixed sodium and potassium carbonates, extract with 1:3 hydrochloric acid and dehydrate in the usual way to remove silica. In the latter case add 1 cc. of concentrated sulfuric acid and heat to sulfur trioxide fumes to remove chlorides. Nearly neutralize the final solution, dilute to a known volume and use an aliquot as a sample.

*Hydrochloric Acid Extracts of Clay Soils.*<sup>36</sup> Boil 20 grams of soil for 5 minutes with 70 cc. of concentrated hydrochloric acid and allow to digest on a water bath for 48 hours. Add water, filter, wash and dilute to 250 cc. Treat 15 cc. of this extract with 0.5 cc. of 20 per cent sodium permanganate solution and heat on a sand bath for 15 minutes. The liquid should show no precipitated manganese dioxide. Cool, dilute to about 30 cc. and add 6 cc. of 10 per cent potassium ferrocyanide solution. This precipitates iron. Add 5 cc. of 10 per cent manganese sulfate solution and shake frequently. After 1 hour carefully add 1:1 ammonium hydroxide until the blue color just turns to purple. Add 3.5 cc. of 2 N sulfuric acid and transfer to a 100 cc. volumetric flask. Dilute to volume and filter. Use a suitable aliquot of the filtrate.

Extraction with N nitric acid has also been recommended as accurate to 5 per cent.<sup>37</sup>

*Citric Acid Extracts of Soils Low in Calcium.*<sup>38</sup> Shake 10 grams of soil with 100 cc. of 1 per cent citric acid solution for 1 hour. A correction factor has to be applied to high-calcium soils. Repeat for 1 hour the next day. Filter or centrifuge and add 10 cc. of 40 per cent sulfuric acid to 5 cc. of the soil solution. Oxidize the citric acid with 0.1 per cent potassium permanganate solution until a faint permanent pink color is obtained. Add 3 per cent hydrogen peroxide to decolorize the permanganate. Digest on a water bath for one-half hour and transfer to a 100 cc. flask. Add 4 drops of o-dinitrophenol as indicator and neutralize with 1:4 ammonium hydroxide. Cool and use as sample by the stannous chloride reduction method.

*Citric Acid Extracts of Clay Soils.*<sup>36</sup> Shake 25 grams of soil for 24 hours in a 500 cc. bottle with 250 cc. of 1 per cent citric acid solution,

<sup>35</sup> P. Urech, *Z. anal. Chem.* 92, 81-6 (1933).

<sup>36</sup> R. G. Warren and A. J. Pugh, *J. Agr. Sci.* 20, 532-40 (1930).

<sup>37</sup> Laszlo Muller, *Kiserlatugyi Kozlemenek*, 34, 77-80 (1931).

<sup>38</sup> Antonin Němec, Josef Lanik and Anna Kappová. *Vestník. svazu výzkumných ústavů zemědělských* 5, 18 (1930); *Listy Cukrovar* 49, No. 4, Rozhledy 2; *Z. Pflanzenernähr., Düngung Bodenle.* 18A, 315-23 (1930); *Ibid.* 21A, 231-3 (1931); *Compt. rend.* 191, 69-71 (1930).

with addition of excess citric acid. Filter and treat 75 cc. of the concentrated hydrochloric acid solution. Let stand for 24 hours for manganese dioxide to disappear. Add 4 cc. of 10 per cent potassium permanganate solution with shaking. After 10 minutes allow to stand until the blue color just turns to green and dilute to volume. Filter.

Phosphorus in such citric acid, <sup>45</sup>

pre-estimate the amount of silica which may be present in the blue of the phosphate. On the basis of the concentration of reagent reducing the i. e. a 5 cc. sample

for calcium carbonate present. Kjeldahl flask with 10 cc. of 20 per cent sodium molybdate and heat until the precipitate has formed. Transfer to a 100 cc. flask and add cyanide solution, drop by drop, until the color just disappears. Add 1:1 ammonium hydroxide. Add 1.5 cc. of 2 N sulfuric acid to the filtrate as sample. Phosphorus can be estimated in the filtrate. 0.2 gram per cc. is molybdate reagent the greater the amount of silica and not give the color in the presence of citric acid. Overcome the effect of citric acid by the presence of 0.2-2.0 mg. of molybdate in the results in the

has been criticized <sup>43</sup> as giving lower results than the method believed to be due to carry-over of precipitate.

of organic

#### *Soil Extracts.*<sup>46</sup>

to leave a colorless filtrate. Add fully to plant extracts and to dye.

Add 5 cc. of saturated bromine solution. Add 5 N sodium hydroxide solution until require about 5 drops. When the color has rendered that the color is rendered

50 cc. of the aqueous solution. This will usually give a color should disappear, the

A, 323-9 (1932).  
*Ähr., Düngung Bodenl.* 25A,

<sup>45</sup> Ritchie R. Ward, *Soil Sci.* 35, 85-97 (1932).

<sup>46</sup> P. L. Hibbard, *Ind. Eng. Chem., Anal. Ed.* 4, 283



by the conversion of bromine to hypobromite during its liberation. If desirable to avoid the presence of excess sulfur dioxide boil, or blow air through the solution.

*Plants.*<sup>46a</sup> Ash 2 grams of finely ground plant material. Moisten the cooled ash with concentrated nitric acid and evaporate to dryness. Dissolve the residue in 1.2 cc. of concentrated nitric acid and dilute to about 25 cc. Filter from silica and dilute to 200 cc. Take a suitable aliquot for development of color against a standard of similar concentration.

The usual wet-ashing methods may also be used but the final aliquot taken for development must not contain more than 18 mg. of sulfuric acid with 0.025–0.1 mg. of phosphorus pentoxide. The method was originated for development of color by stannous chloride.

*Feeds, Feces and Fertilizers.*<sup>32a</sup> Digest 1–4 gram samples with 15–20 cc. of nitric acid. Proceed with perchloric acid ashing as usual<sup>46b</sup> except to add only 1 cc. or, if necessary, 2 cc. of 70 per cent perchloric acid. Occasionally a second addition of nitric acid also proves desirable.

*Cane Juice.*<sup>47</sup> Evaporate and ignite 20 cc. of cane juice with 4 drops of 10 per cent calcium acetate solution. Extract the ash with 10 cc. of 1:9 sulfuric acid and filter into a 100 cc. flask. Wash the residue and filter. Wash until free of acid and dilute to volume. Use a suitable aliquot as sample for comparison with a standard of very nearly the same concentration.

*Sugar. Total Phosphorus.*<sup>48</sup> Mix 5 grams of sugar in a platinum dish with 0.2 gram of anhydrous sodium and potassium carbonates. Char carefully over a free flame and finally incinerate in a muffle furnace below a temperature at which the ash will fuse. Cool and dissolve the ash in 1 cc. of 1:1 nitric acid. Render the silica insoluble by evaporating to dryness on the steam bath. Add 0.5 cc. of concentrated nitric acid and again evaporate to dryness on the steam bath. Take up with 5 cc. of water and filter: at once to remove silica, catching the filtrate in a 100 cc. Nessler tube. Wash the filter until free of acid and use this solution as sample. The Briggs modification of the Bell and Doisy method was used by Byall and Ambler.

*Sugar. Organic Phosphorus.* Subtract the value for inorganic phosphorus from that for total phosphorus, both to be expressed in comparable terms. Note the difference in weight of sample.

<sup>46a</sup> J. Tischer, *Z. Pflanzenernähr., Düngung Bodenb.* 33A, 192-242 (1934).

<sup>46b</sup> See pp. 498-9.

<sup>47</sup> B. E. Beater, *Proc. S. African Sugar Tech. Assoc. 7th Ann. Congr.* 1933, 87-93.

<sup>48</sup> S. Byall and J. A. Ambler, *Ind. Eng. Chem., Anal. Ed.* 3, 136-7 (1931).

*Organic Compounds.*<sup>49</sup> Take a sample sufficient to contain 8 mg. of phosphorus. Dissolve in alcohol and dilute to 100 cc. Mix and pipet 1 cc. into a Pyrex tube with 2.5 cc. of 0.525 *N* sulfuric acid. Heat until charring appears. Cool, add 4 drops of 30 per cent hydrogen peroxide and heat again. Finally add 2 drops more of 30 per cent hydrogen peroxide and evaporate to 0.5 cc. Add 2 cc. of water and evaporate to 0.5 cc. Repeat. Dissolve in 5 cc. of water and dilute to about 10 cc.

*Orthophosphoric Acid in Other Acids Containing Phosphorus.*<sup>50</sup> Other acids of phosphorus such as metaphosphoric and pyrophosphoric do not give the reaction. Suitably diluted they may therefore be used as sample, and their rate of hydration to orthophosphoric acid estimated. The method of reduction by aminonaphthol sulfonic acid has been applied.<sup>50a</sup>

*Aluminum.* Aluminum in the form of borings can be dissolved in hydrochloric acid in a stream of hydrogen. The phosphorus is evolved as phosphine. The hydrogen when burned in air converts the phosphine to phosphoric acid, which is condensed with the evolved water.<sup>51</sup> Special apparatus has been designed, applying this principle to a micro determination.<sup>52</sup> Silicic acid does not interfere.<sup>53</sup> Agreement within 5 per cent is obtained on 0.005 to 0.0008 per cent.

The accompanying Figure 79 shows the apparatus required. Tubes W and F are used to regulate the stream of hydrogen to 160 cc. per minute. Flask Z is the container for the sample of aluminum. It is fitted with a safety tube T. Hydrochloric acid from the dropping funnel falls on the aluminum. The water condenser and flask V remove any liquid passing over. The phosphine evolved is swept along by the stream of hydrogen and passes out through a small quartz capillary, 3 cm. long and 0.5 mm. in diameter. At the outlet of this capillary the gases are ignited by means of an electric spark passing between platinum wires. By regulating the stream of hydrogen, the height of the flame is kept at 6–8 mm., so that the tip almost touches the condensing vessel. The inner condensing vessel in cylinder C leaves a passage of 2 mm. between it and the outer cylinder. The lower end of this condenser is drawn out to a tip from which condensed liquid drops into a test tube below. Cylinder C has a similar tip, with a tube beneath to catch condensed liquid.

<sup>49</sup> Walter C. Davies and Daniel Richard Davies, *J. Chem. Soc.* 1931, 1207-11.

<sup>50</sup> A. Dunajew, *Z. anal. Chem.* 80, 252-63 (1930).

<sup>50a</sup> Kazimierz Boratyuski, *Polish Agr. Forestal Ann.* 34, 95-106 (1935).

<sup>51</sup> K. Steinhauser, *Z. anal. Chem.* 81, 433-8 (1930).

<sup>52</sup> W. D. Treadwell and J. Hartnagel, *Helv. chim. Acta* 15, 1023-9 (1932).

<sup>53</sup> Cf. K. Steinhauser and J. Stadler, *Z. anal. Chem.* 91, 165-70 (1932).

The outer cylinder is about 25 cm. long and 5 cm. in diameter. In order to supply the oxygen necessary to support a flame, an outlet from C leads through a spiral washing flask A to a suction pump. By this means 1 liter of air per minute is drawn through cylinder C. Examination of an alkaline solution placed in A showed no trace of phosphorus at the end of 15 successive determinations.

Weigh into flask Z 0.1 to 1 gram of fine aluminum borings corresponding to 0.001 to 0.06 mg. of phosphorus. Turn on the hydrogen. It is well to have a stopcock in the line shortly before reaching the quartz outlet. When all air has been flushed out of the equipment, start the

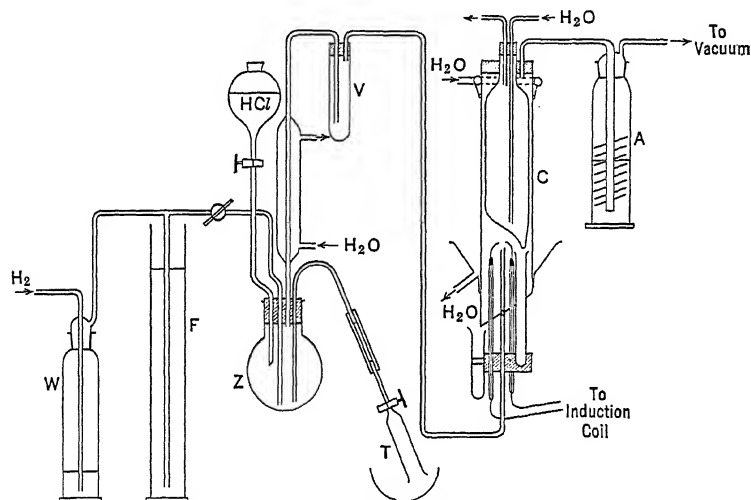


FIG. 79

Apparatus for Evolution of Phosphine and Combustion to Phosphoric Acid

ignition sparking. Start the water condensers and air current. Slowly drop 10 per cent hydrochloric acid on the sample. It should require from 20 to 40 minutes for the sample of aluminum to dissolve. Let the hydrogen pass through 15 minutes longer after solution is complete. Then fill flask Z to the neck with 10 per cent hydrochloric acid.

Wash the condensate out of the ignition jar and unite with that from the tubes in a platinum dish. Add 1 to 2 drops of 1:4 sulfuric acid and evaporate to about 10 cc. Use this as sample. As developed, the method uses the stannous chloride reduction procedure.

*Iron and Steel.* Use the method described above for aluminum. Use

a 0.2 gram sample containing about 0.015 gram of phosphorus, or modify according to phosphorus content.

*Iron and Steel. Alternative Procedure.*<sup>53a</sup> Dissolve 1 gram of iron or steel in 50 cc. of 1:1 nitric acid and oxidize with 5 cc. of 3 per cent potassium permanganate solution. At the same time treat an amount of standard containing a similar amount of phosphorus in the same way. Boil for a few minutes and add 5 cc. of saturated ammonium oxalate solution. The standard may require slightly more. Boil for 1 to 2 minutes to dissolve manganese dioxide and expel carbon dioxide. Dilute to 100 cc. and use an aliquot of this as sample. Extract the sample with an equal volume of ether in several portions to remove the major part of the iron. Add an amount of iron to the standard similar to that remaining in the sample and treat sample and standard at the same time to develop the color. The method was originated for stannous chloride development of color.

*Ashing—Dry Ashing.* This method without additions, or with the addition of magnesium nitrate or sodium carbonate, is occasionally used. It is generally considered unreliable. One cc. of a 10 per cent solution of either is sufficient for a 5 cc. sample. When ashed with a fusion mixture of sodium carbonate and potassium nitrate the acidified fusion must be boiled for a short time to remove nitrous acid formed by reduction of the nitrate. A modification<sup>54</sup> provides for ignition of the sample mixed with 2 parts by weight of calcium carbonate and covered with a layer of calcium carbonate. Good results for gravimetric work have been reported in comparison against magnesium nitrate ashing methods.

For magnesium nitrate ashing,<sup>54a</sup> pipet an aliquot of sample containing 0.05–0.15 mg. of phosphorus into a beaker and add 2 cc. of a 50 per cent magnesium nitrate solution. Evaporate on a hot plate until a portion of the ash becomes white. Usually 15–20 minutes are required after full temperature is reached. If a portion of the ash becomes charred, cool, add a few cc. of water and a drop of concentrated nitric acid and heat again. Heat over a free flame or in a muffle until the magnesium nitrate is decomposed. This should leave a white ash without free carbon. Dissolve the ash in 10–20 cc. of water by heating. Cool and neutralize to Congo red paper with concentrated ammonium hydroxide. Use this solu-

<sup>53a</sup> S. B. Shneerson, *Zavodskaya Lab.* 3, 21-4 (1934).

<sup>54</sup> B. W. Hawk and E. E. De Turk, *Ind. Eng. Chem., Anal. Ed.* 4, 111-2 (1932).

<sup>54a</sup> R. R. Roepke, *Ind. Eng. Chem., Anal. Ed.* 7, 78 (1935).

tion as sample or transfer to a volumetric flask, dilute to volume and take an aliquot as sample.

*Acid Ashing.* Ashing of blood and tissues by digestion with various mixtures of nitric and sulfuric acids<sup>55</sup> is probably the most widely used and most criticized method. It has been found satisfactory by numerous investigators.<sup>56-63</sup> In nearly every case caution against heating the tube at the meniscus, overheating which allows the escape of too great a quantity of fumes, and failure to remove the last trace of nitric acid has been made. Hartwell, Bosworth and Kellogg<sup>64</sup> found results about 3 per cent too high by the acid method and also found it more difficult to obtain checks. They prefer the use of magnesium nitrate. Hillebrand and Lundell<sup>65</sup> find loss due to evaporation to complete expulsion of sulfuric acid, evaporation at a temperature over 200° and unduly prolonged evaporation at over 150°. Richter-Quittner<sup>66</sup> warns against loss by volatilization. Lebediantzev<sup>67</sup> has pointed out that such losses occur. Martland and Robison<sup>68</sup> found in heating in round bottom flasks loss of phosphorus by combination with the glass. Baumann<sup>69</sup> has detected phosphoric acid in the vapors from acid ashing, showing losses of 2 to 15 per cent. Raper,<sup>70</sup> and Taylor and Miller,<sup>71</sup> recommend dry ashing in preference to wet. The method has also been used<sup>72</sup> with hydrogen peroxide to complete the reaction.

To the sample in a suitable tube add 5 cc. of 1:2 sulfuric acid and a piece of quartz or a couple of glass beads to prevent bumping. Heat in a paraffine bath at 120° until all water has evaporated. Increase the

<sup>55</sup> Albert Neumann, *Z. physiol. Chem.* 37, 129 (1902-3); *Ibid.* 43, 35 (1904).

<sup>56</sup> Isidor Greenwald, *J. Biol. Chem.* 21, 29 (1915).

<sup>57</sup> W. R. Bloor, *Ibid.* 22, 133 (1915); *Ibid.* 36, 33 (1918).

<sup>58</sup> Richard D. Bell and Edward A. Doisy, *Ibid.* 44, 55 (1920).

<sup>59</sup> F. S. Randles and Arthur Knudson, *Ibid.* 53, 53 (1922).

<sup>60</sup> John C. Whitehorn, *Ibid.* 62, 133 (1924).

<sup>61</sup> Cyrus H. Fiske and Y. Subbarow, *Ibid.* 66, 375-400 (1925).

<sup>62</sup> Herman Minglais, *Bull. soc. chim. biol.* 9, 540-53 (1927).

<sup>63</sup> Michael Machelboeuf and Genevieve Zwilling, *Ibid.* 9, 697-9 (1927).

<sup>64</sup> B. L. Hartwell, A. W. Bosworth and J. W. Kellogg, *J. Am. Chem. Soc.* 27, 240 (1905).

<sup>65</sup> W. F. Hillebrand and G. E. F. Lundell, *J. Am. Chem. Soc.* 42, 2609 (1920).

<sup>66</sup> M. Richter-Quittner, *Biochem. Z.* 126, 97 (1921).

<sup>67</sup> A. Lebediantzev, *Russ. J. Agr. exp.* 18, 14 (1917).

<sup>68</sup> M. Martland and R. Robison, *Biochem. J.* 18, 765 (1924).

<sup>69</sup> E. J. Baumann, *J. Biol. Chem.* 59, 667 (1924).

<sup>70</sup> A. S. Raper, *Biochem. J.* 8, 649 (1914).

<sup>71</sup> A. E. Taylor and C. W. Miller, *J. Biol. Chem.* 18, 215 (1914).

<sup>72</sup> J. H. Roe, O. J. Irish and J. I. Boyd, *J. Biol. Chem.* 67, 579 (1926).

temperature to 180° or until charring begins. Heating the tube on an electric hot plate inclined at a slight angle is almost equally good and a micro burner may be used with but little loss of accuracy. The tube should be about 2 cm. above the flame of the burner and care should be taken that the meniscus is never heated. As soon as charring begins a drop of concentrated nitric acid is run down the wall of the test tube and is usually sufficient to cause decolorization. If the color does not disappear at once add more nitric acid drop by drop until decolorized. Nothing is to be gained by the use of great excess.

The original Neumann procedure calls for equal parts of nitric and sulfuric acid. Bloor<sup>57</sup> found it necessary to remove the last trace of nitric acid by addition of a drop of sugar solution. This can be avoided by using the minimum amount as above.

When decolorized, boil gently for 1 minute to complete the ashing and to drive off the last traces of nitric and nitrous acids. At this stage the fumes of sulfur trioxide should fill the tube and a few wisps just escape. The loss of large amounts of fumes will give low results. After cooling, dilute with water and develop the color. Acidify the standard for comparison with 5 cc. of 1:2 sulfuric acid so that color will be developed under parallel conditions.

*Perchloric Acid Ashing.* This form of acid ashing permits great reduction in the time of ashing.<sup>73a</sup> Losses of potassium and phosphorus do not occur.<sup>73b</sup> It has been widely applied<sup>73c,d,e</sup> to plant and animal substances. The perchloric acid serves to dehydrate silica.<sup>73f</sup> The residual acid has neither oxidizing nor reducing properties. Care must be used to pretreat organic samples with nitric acid to avoid the explosive properties of perchloric acid. If the sample is high in fat several pretreatments may be needed before proceeding with the destruction described below. The resulting solution is suitable for estimation of calcium, potassium and magnesium as well as phosphorus.

Transfer a sample of suitable size to a 500 cc. Kjeldahl flask. Add 20-30 cc. of concentrated nitric acid and heat over asbestos gauze. Boil gently with frequent swirling until the contents become swollen and then

<sup>73a</sup> H. W. Gerritz, *Ind. Eng. Chem., Anal. Ed.* 7, 167-8 (1935).

<sup>73b</sup> J. E. Giesecking, H. J. Snider and C. A. Getz, *Ibid.* 7, 185-6 (1935).

<sup>73c</sup> E. Kahane, "L'action de l'acide perchlorique sur les matières organiques." Hermann et Cie, Paris (1934).

<sup>73d</sup> L. Lematter, G. Boinot, E. Kahane and M. Kahane, *Compt. rend.* 192, 1459 (1931).

<sup>73e</sup> O. B. Winter and O. D. Bird, *J. Am. Chem. Soc.* 51, 2964 (1929).

<sup>73f</sup> H. H. Willard and W. E. Cake, *Ibid.* 42, 2208 (1920).

## BY REDUCTION OF THE PHOSPHOMOLYBDATE.

pass into fine suspension or solution. This usually takes 30 to 45 minutes. Avoid heating to dryness. Add 10 cc. of 70 per cent perchloric acid and heat over a low free flame. Only a fine point of the flame should touch the flask and the contents should just boil. Higher temperatures will cause loss of perchloric acid without material acceleration of the rate of digestion.

When fuming starts adjust the flame so that only a trace of the perchloric acid fumes reach the upper part of the neck of the flask. Continue until colorless or faintly yellow. Let cool somewhat and add 50 cc. of distilled water. The vigorous boiling due to heat of dilution drives off the remaining nitric oxide fumes to leave a clear solution. Filter the solution into a volumetric flask and rinse the Kjeldahl flask with distilled water. When cool dilute to volume.

*Peroxide Ashing.* To both sample and standard add 1 cc. of 1:2 sulfuric acid. Heat as discussed under acid ashing. Evaporate the water and continue heating. Some sulfuric acid should be volatilized but no fumes should be driven from the tube. At the first signs of darkening add 30 per cent hydrogen peroxide from a capillary pipet in small drops, continuing the addition from time to time until no more darkening occurs.

When the last trace of color has been destroyed increase the heat until the tubes are half full of fumes. Maintain at this temperature for 6 minutes to destroy all traces of excess oxidizing agent. Excess of hydrogen peroxide is normally destroyed in 3 minutes in boiling sulfuric acid.<sup>73b</sup> The time required for complete expulsion of excess oxidizing agent is much less than when nitric acid is used. Cool, rinse the sides of the tubes with water and dilute to about 10 cc. Boil for 10 minutes to convert all phosphorus to orthophosphoric acid. Cool sample and standard together in a bath of cold water and develop the color.

If necessary for purification distil 100 cc. of C.P. 30 per cent hydrogen peroxide at a time under vacuum, keeping the temperature below 60° at all times. The entire distillate is used. Handle it with great caution as it produces severe burns. Wash all apparatus thoroughly with distilled water as the solution must contain no heavy metals. The peroxide must never come into contact with rough surfaces or organic matter. This involves insertion of the side arm of the distilling flask far into the condenser. Use a capillary tube to prevent bumping. According to Wolfenstein<sup>74</sup> distillation at 65 mm. between 71° and 81° yields 44 per cent hydrogen peroxide.

<sup>73b</sup> David Glick, *J. Lab. Clin. Med.* 19, 1912-13 (1934).

<sup>74</sup> Richard Wolfenstein, *Ber.* 27, 3311 (1894).

**Procedure**—For this reaction the first procedure was to separate the molybdate precipitate, wash, dissolve in alkali and reduce. The method was first proposed by Osmund<sup>75</sup> using stannous chloride as reducing agent. Taylor and Miller<sup>71</sup> proposed the use of phenylhydrazine for biological work. As both of these reducing agents under the conditions used molybdic acid, the method was indirect by estimation with the phosphorus and so has obvious advantages is reduced by benzidrin

Wu<sup>81</sup> use of acid suggested agent. A

id. The reduction is localized in one or two atoms of the compound. The blue product which results is a negative colloid obtained only in solution. It also exists as a crystalloid. The hydrogel has the formula  $\text{Mo}_3\text{O}_8\cdot\text{H}_2\text{O}$ .<sup>84</sup> Deniges<sup>85</sup> introduced stannous chloride as a reducing agent under conditions which reduced only the phosphomolybdate. This has been greatly improved and is probably the best reducing agent for the purpose.<sup>86-94</sup> Six moles of stannous chloride per mole of

<sup>75</sup> F. Osmund, *Chem. News* 56, 160 (1887); *Bull.*

<sup>77</sup> F. Feigl, *Z. anal. Chem.* 74, 386-92 (1928); *II*

<sup>78</sup> H. Leitmeier, *Mikrochemie* 6, 144-8 (1928).

<sup>79</sup> G. A. Markova, *Udobrenie i. Urozhai* 3, 832-6 (

<sup>80</sup> N. Passerini, *Gazz. chim. ital.* 41, I, 182 (1911).

<sup>81</sup> Hsein Wu, *J. Biol. Chem.* 43, 218 (1920).

<sup>82</sup> L. Losana, *Giorn. chim. ind. applicata* 4, 60-2

<sup>83</sup> Hsein Wu, *J. Biol. Chem.* 43, 211 (1920).

<sup>84</sup> Lloyd A. Munro, *Proc. Trans. Nova* 1924 (192

<sup>85</sup> G. Deniges, *Compt. rend.* 171, 805 (1921); *Compt. rend.* 185, 777-9 (1927).

<sup>86</sup> Emil Truog and A. H. Meyer, *Ind. Eng. Chem., Anal. Ed.* 1, 136-9 (1929).

<sup>87</sup> T. Kuttner and H. R. Cohen, *J. Biol. Chem.* 75, 517-31 (1927).

<sup>88</sup> F. W. Parker and J. F. Fudge, *Soil Sci.* 24, 109-17 (1927).

<sup>90</sup> W. R. G. Atkins, *J. Agr. Sci.* 14, 192-7 (1924).

<sup>91</sup> B. E. Benter, *Proc. S. African Sugar Tech. Assoc. 7th Ann. Cong.* 1933, 87-93.

<sup>92</sup> Giuseppe Gherardini and Maria Brasi, *Diagnostica tec. lab. (Napoli) Riv. mensile* 1, 1043-50 (1930).

<sup>93</sup> Theodore Kuttner and Louis Lichenstein, *J. Biol. Chem.* 86, 671-6 (1930); *Ibid.* 70 (1932).

<sup>94</sup> H. Zinzedz, *Udobrenie i. Urozhai* 3, 827-32 (1931).



phosphorus pentoxide are necessary.<sup>95</sup> Six of the 12 molybdenum atoms in phosphomolybdic acid can be reduced. Hydroquinone has also been used.<sup>98</sup> The color changes rather rapidly and with an adequate amount of hydroquinone gives a relatively large blank determination. The method has had numerous modifications designed to produce a more permanent color. The blue alkaline solution has been modified to the green acid one by Briggs<sup>97</sup> and other changes have been made.

A 3.128 per cent solution of *p*-methylaminophenol sulfate, sold as Elon, has been used to replace a 1 per cent solution of hydroquinone.<sup>98</sup> The best reducing agents have been stated to be those with —OH and —NH<sub>2</sub> groups in the para position to each other.<sup>99</sup> The procedure has been used as a micro method on 0.2 cc. of serum, as from infants.<sup>100</sup>

An improvement in the procedure has been the introduction<sup>61</sup> of another reducing agent, 1, 2, 4-aminonaphthol sulfonic acid, which develops the color many times as quickly as the reagent of Bell and Doisy. Isomeric naphthol sulfonic acids do not develop the color as rapidly but some give a deeper color.<sup>100a</sup> One investigator has used molybdic acid quantitatively reduced with molybdenum.<sup>101</sup> The method has been extensively applied.<sup>90, 102b-m</sup> The complex from reduction of molybdenum tri-

<sup>95</sup> Stina Gripenberg, *Beret. 18th Skand. Naturforskermode (Copenhagen)* 1929, 293-8.

<sup>97</sup> A. P. Briggs, *J. Biol. Chem.* 53, 13-16 (1922); *Ibid.* 59, 255 (1924).

<sup>98</sup> G. van der Luigen, *Analyst* 58, 755-6 (1933).

<sup>99</sup> Ernst Tschopp and Emilio Tschopp, *Helv. Chim. Acta* 15, 793-809 (1932).

<sup>100</sup> E. Freudenberg, *Z. Kinderheilk.* 51, 267-72 (1931).

<sup>100a</sup> Ch. Zinzadze, *Chimie & Industrie* 27, 841-3 (1932).

<sup>101</sup> Ch. Zinzadze, *Ann. agron.* 1, 321-36 (1931); *Z. Pflanzenernähr., Düngung Bodenk.* 16A, 129-84 (1930); *Ind. Eng. Chem., Anal. Ed.* 7, 227-30 (1935).

<sup>103</sup> B. Vasarhelyi, *Mikrochem. Pregl. Festschr.* 1929, 329-37.

<sup>103b</sup> A. H. Lewis and F. B. Marmoy, *J. Soc. Chem. Ind.* 52, 177-82T (1933).

<sup>103c</sup> F. Alten, H. Weiland and H. Loofmann, *Z. Pflanzenernähr., Düngung Bodenk.* 32A, 33-50 (1933).

<sup>103d</sup> R. B. Deemer and J. A. Schricker, *J. Assoc. Official Agr. Chem.* 16, 226-32 (1933).

<sup>103e</sup> C. Dreyspring and W. Heinz, *Z. Pflanzenernähr., Düngung Bodenk.* 35A, 362-74 (1934).

<sup>103f</sup> D. Feher, *Phosphorsäure* 3, 429-36; *Ibid.* 4, 508-29 (1933); *Z. Pflanzenernähr., Düngung Bodenk.* 33A, 320-35 (1934).

<sup>103g</sup> H. W. Gerritz, *Ind. Eng. Chem., Anal. Ed.* 7, 116-8 (1935).

<sup>103h</sup> A. Guyer and A. Likiernik, *Helv. Chim. Acta* 16, 1033-44 (1933).

<sup>103i</sup> A. H. K. Petrie, *Australian J. Exp. Biol. Med. Sci.* 11, 25-34 (1933).

<sup>103j</sup> W. O. Robinson, H. C. Dudley, K. T. Williams and H. G. Byers, *Ind. Eng. Chem., Anal. Ed.* 6, 274-6 (1934).

<sup>103k</sup> W. D. Treadwell and J. Hartnagel, *Helv. Chim. Acta* 15, 1023-9 (1932).

<sup>103m</sup> R. R. Ward, *Soil Sci.* 24, 85-97 (1933).

oxide,  $\text{MoO}_2 \cdot 4\text{MoO}_3$ , is unstable and destroyed on aqueous dilution while the compound from phosphorus  $(\text{MoO}_2 \cdot 4\text{MoO}_3)_2\text{H}_3\text{PO}_4$  and the similar one from arsenic are stable. Increasing acidity eliminates the effect of silica. Sodium bisulfite eliminates effects from arsenates,<sup>103n,o</sup> nitrates and ferric iron.

The development of the color depends on the pH of the solution and on the time of reaction.<sup>104</sup> Oxalic and citric acids retard the reduction. Nitric, hydrochloric, sulfuric and trichloroacetic acids have little effect. Acetic acid shows practically none. If 5 per cent of sulfuric acid is present in samples and standards, the color is modified but then silicic acid will not react.<sup>105</sup>

The use of large amounts of some acids is undesirable as neutralization of the excess produces a high salt concentration. Chlorides and nitrates interfere, sulfates do not.<sup>106</sup> Possible interference by substantial amounts of pyrophosphoric, glycerophosphoric, citric, oxalic, pyruvic, tartaric, malic, lactic and glycolic acids is avoided by alteration of the amount of molybdate reagent, based on experience.<sup>107</sup> All but the first two acids can also be removed by ashing.

Arsenomolybdates are reduced under the same conditions but a technique has been developed by which phosphorus and arsenic can both be determined, the arsenic being reduced before addition of ammonium molybdate.

*Hydrazine Sulfate Reduction.*<sup>109</sup> Since the reducing agent also reduces ammonium molybdate the phosphomolybdate must first be separated and washed.

Concentrate the sample to 1–2 cc. and transfer to a graduated centrifuge tube with 30 per cent ammonium nitrate solution. Add 1 cc. of 1:2 nitric acid and dilute with 30 per cent ammonium nitrate solution to 10 cc. Heat to 50 to 60° and add 1.2 cc. of 5 per cent neutral ammonium molybdate solution. Mix, let stand 10 minutes and centrifuge 2 to 3 minutes. Pour off the clear liquid and add 5 cc. of 30 per cent ammonium nitrate solution. Break up the precipitate with a stirring rod and again centrifuge. Pour off the clear wash solution. Wash the precipitate from the tube into a 100 cc. volumetric flask with 20 cc. of colorless 2 per cent

<sup>103n</sup> N. S. Tanonief and Ch. N. Potschinok, *Z. anal. Chem.* 88, 271-8 (1932).

<sup>103o</sup> L. B. Pett, *Biochem. J.* 27, 1672-6 (1933).

<sup>104</sup> Laszlo Urbanek, *Mesőgazdasági Kutatások* 4, 39-59, 163-73 (1931).

<sup>105</sup> E. W. Scarritt, *Ind. Eng. Chem., Anal. Ed.* 3, 23 (1931).

<sup>106</sup> John Munsell, *Proc. Soc. Exptl. Biol. Med.* 29, 828-9 (1932).

<sup>107</sup> Daniel R. Davies and Walter C. Davies, *Biochem. J.* 26, 2046-55 (1932).

<sup>109</sup> E. Riegler, *Bull. acad. sci. Roumanie* 2, 272 (1914).

hydrazine sulfate solution. Heat 10 minutes at 50° and let cool. When cool dilute to volume and mix.

Compare with a standard prepared from a suitable volume of solution containing 0.1 mg. of phosphorus pentoxide per cc. which has been treated in the same way. The standard should have been treated at about the same time although the color is reasonably stable.

A 2 per cent sodium thiosulfate solution may also be used for reduction of the hot solution of phosphomolybdate.<sup>82</sup>

*Stannous Chloride Reduction.* Less than 700 p.p.m. of silica does not interfere if the acid is added first to repress the silicic acid.<sup>111</sup> Ferric ion over 4-6 p.p.m. decreases the intensity of color and produces a greenish tint. Ferrous ion produces no undesirable effect. In the presence of iron reduce in a Jones reductor with metallic cadmium and then proceed with the determination. Aluminum and manganese do not interfere in reasonable amounts. Nitrate up to 100 p.p.m. has no effect, 200 p.p.m. reduces the color 10 per cent. Calcium and magnesium up to 1000 p.p.m. have no effect.

Arsenic Absent. To the solution of sample add sufficient alkali to neutralize. Adjust the temperature to 25°. Add 4 cc. of 10 *N* sulfuric acid and 4 cc. of 2.5 per cent ammonium molybdate solution. Mix well, add 6 drops of 2.5 per cent solution of stannous chloride in 1:9 hydrochloric acid. Mix well, dilute to 100 cc. with water at 25° and compare with a standard similarly treated. The color develops at once, and starts to fade in about 10 minutes. It may be regenerated, when fading starts, by addition of another drop of stannous chloride solution, and this may be repeated at 10 minute intervals for about 1 hour.

The maximum intensity of color is not developed if the acidity is not correct. Ferric ion affects the color markedly. The stannous chloride deteriorates on standing, even though protected by a layer of mineral oil.<sup>112</sup> Minor variations in its strength are unimportant. Temperature must be standardized as it has a measurable effect on the rate of development of the blue color.

Arsenic Present. Pass hydrogen sulfide through the solution to reduce arsenic completely. Boil off excess hydrogen sulfide and add paper pulp. Mix well and filter. Proceed as for the solution with arsenic absent.

*Alternative Stannous Chloride Reduction.*<sup>112a</sup> Prepare a molybdic

<sup>111</sup> H. D. Chapman, *Soil Sci.* 33, 125-34 (1932).

<sup>112</sup> H. D. Chapman, *Ind. Eng. Chem., Anal. Ed.* 3, 282-4 (1931).

<sup>112a</sup> Ch. Zinzadze, *Ind. Eng. Chem., Anal. Ed.* 7, 227-30 (1935).

acid reagent as follows: Transfer 101 cc. of exactly 25 *N* sulfuric acid to a 500 cc. flask. Add molybdic acid containing exactly 4.01 grams of molybdic anhydride. Boil very gently with occasional swirling until solution is complete. Avoid sulfur trioxide fumes. Cool to room temperature and pour slowly into a 1 liter flask containing about 900 cc. of water. Dilute to about 998 cc., cool, dilute to volume and mix. Pipet 10 cc. accurately and dilute to 100 cc. Titrate 10 cc. of this solution with 0.1 *N* sodium hydroxide solution. It should require 24.9–25.1 cc. of the hydroxide. This reagent keeps indefinitely if protected from contamination.

Pipet 0.5–15 cc. of sample solution containing 0.01–0.3 mg. of phosphorus pentoxide into a 50 cc. flask. Pipet an equivalent volume of standard into another flask and add to it the substances known to be present in the sample. Add 5 drops of saturated aqueous  $\alpha$ -dinitrophenol (2, 4-dinitrophenol) and neutralize to a very faint yellow with 2 per cent sodium bicarbonate solution or with *N* sulfuric acid. Add 5 cc. of *N* sulfuric acid and mix. Add 5 cc. of 8 per cent sodium bisulfite solution, dilute to about 30 cc. and mix. Let stand over night or heat on a steam bath for 1 hour. Cool and add 5 cc. of the molybdic acid reagent to each. Mix and add 5 cc. of a stannous chloride reagent containing 0.16 gram of stannous chloride dihydrate in 200 cc. of 1 per cent gum arabic solution preserved with toluene. Dilute to 50 cc. and compare after 20 minutes. The solution becomes faintly turbid in 3 hours. Standard and reagent must have been prepared simultaneously. This color will not be altered by 2 mg. of ferric oxide or 10 mg. of nitric acid.

*Hydroquinone reduction.* Estimate the approximate acidity of the sample when diluted to 20 cc. according to the method of preparation of the sample. By acid ashing this would be 1.5 *N* and by peroxide ashing 0.3 *N*. The zone of acidity in which this determination is satisfactory is 0.9 to 1.9 *N*. It is therefore desirable to aim at obtaining 1.4 *N*.<sup>72</sup> To attain this add 4 cc. of 1:2 sulfuric acid to the sample and standard from peroxide ashing or 5 cc. to neutral samples.

Dilute both sample and standard to 8 cc. Add 4 cc. of 5 per cent ammonium molybdate solution in 2 *N* sulfuric acid, 2 cc. of 1 per cent hydroquinone solution and 4 cc. of 20 per cent sodium sulfite solution. Dilute each to 20 cc. and compare at the end of one-half hour.

The solutions should stand in the colorimeter for at least 5 minutes before comparison.<sup>59</sup> The presence of excessive amounts of salt or acid interferes,<sup>61,115</sup> with the rate of development of color. The hydroquinone solution will keep indefinitely if made 2 *N* with sulfuric acid. Bell and

<sup>115</sup> C. Rimington, *Biochem. J.* 18, 1279 (1924).

Doisy have stated that this fading of color is at a different rate in the colorimeter cup and in the flask. The difference was later shown to be due to bubbles of sulfur dioxide which must be allowed to separate. The color of the blank is such as to preclude determination of less than 0.02 mg. of phosphorus by this method.<sup>60</sup> Dilution is in a linear ratio so that deeply colored samples may be diluted to a suitable range and compared. The phosphorus content of the sample and standard compared should not differ by more than 30 per cent. If the sample contains trichloroacetic acid it should also be added to the standard.<sup>118</sup> While this is not always necessary it is always advisable.

In another modification<sup>119</sup> a 5 cc. sample is treated with 3 cc. of water, 1 cc. of 5 per cent sodium molybdate solution in 1:1 sulfuric acid and 1 cc. of a solution containing 15 per cent of sodium bisulfite and 0.5 per cent of hydroquinone. It is then mixed, loosely stoppered and heated simultaneously with a standard in a boiling water bath. The color is stated to be three times as intense as by the Briggs procedure.

*Aminonaphthol Sulfonic acid reduction.*<sup>61</sup> The production of 1, 2, 4-aminonaphthol sulfonic acid has been described by Folin<sup>121</sup> but requires further washing with alcohol until the washings are colorless. It may be purchased in a partially purified form which should be recrystallized. Heat 1 liter of water to 90° and dissolve in it 150 cc. of sodium bisulfite and 10 grams of crystallized sodium sulfite. Add 15 grams of the crude aminonaphthol sulfonic acid and stir until all but a trace of impurity has dissolved. Filter the hot solution, cool under the tap and add 10 cc. of concentrated hydrochloric acid to reprecipitate the sulfonic acid. Mix well and filter with suction. Wash with 300 cc. of distilled water and finally with alcohol until the washings are colorless. Air-dry with minimum exposure to light and keep in a brown bottle.

Prepare a 0.25 per cent solution as follows. Dissolve about 0.5 gram of the dry powder in 195 cc. of 15 per cent sodium bisulfite solution, add 5 cc. of 20 per cent clear sodium sulfite solution, stopper and shake. If the bisulfite is old the acid may not completely dissolve and in that case add more sulfite solution, 1 cc. at a time, shaking after each addition until the solution is clear. Dilute to approximately 250 cc. If not exposed to air the reagent will keep about two weeks. The stability decreases as more sulfite is added.

For determination transfer the sample to a 25 cc. calibrated tube and

<sup>118</sup> R. V. Stanford and A. H. Wheatley, *Biochem. J.* 19, 697 (1925).

<sup>119</sup> S. R. Benedict and R. C. Theis, *J. Biol. Chem.* 61, 63 (1924).

<sup>121</sup> Otto Folin, *J. Biol. Chem.* 51, 386 (1922).

dilute to about 10 cc. Add 1 cc. of 5 per cent ammonium molybdate, 1 cc. of 1:3 sulfuric acid and 1 cc. of the reagent. In the case of the filtrates precipitated by trichloroacetic acid, add only 0.5 cc. of 1:3 sulfuric acid to the sample, the difference compensating for the other acid present. Treat the standard exactly the same including salt present in the sample. It is essential that standard be nearly the same. Keep the flasks at 37° for 5 minutes to develop the full color. The best results are obtained when 0.2-0.4 mg. of phosphorus pentoxide is present.<sup>122</sup>

l-Ascorbic acid has been found equally effective.<sup>87</sup> A labile form of ascorbic acid present in muscle was demonstrated by the following method. The sample must be ashed.<sup>1</sup>

. Make up to volume.

solution.

lute

to about 50 cc. and titrate with 0.1 N potassium dichromate, such as rinsing with water. d. Mix solution I and solution II. The solution

keeps for years in a glass-stoppered bottle.

For use of commercial reagent it is necessary to check on two portions. Dilute 5 cc. to 500 cc. and titrate 10 cc. of this dilution with 0.1 N sodium

<sup>122</sup> L. Jendrassik, *Biochem. Z.* 178, 419-26 (1926)

<sup>124</sup> Cyrus H. Fiske and Y. Subbarow, *Science* 65, 401-3 (1927).

<sup>125</sup> Frances Krasnow, Maxwell Karshan and Laura E. Krajci, *J. Lab. Clin. Med.* 17, 1148-52 (1932).

<sup>126</sup> Earl J. King, *Biochem. J.* 26, 292-7 (1932).

<sup>127</sup> Ruth E. L. Berggren, *J. Biol. Chem.* 95, 461-4 (1932).

hydroxide solution to a phenolphthalein end-point. It should require 24.9–25.1 cc. of hydroxide indicating that it is 25 *N*. Titrate 5 cc. diluted to about 50 cc., with 0.1 *N* potassium permanganate solution. It should require 4.9 cc. to 5.1 cc. If it falls below 4.9 cc. discard the reagent. Repeat this check determination monthly.

For use dilute a measured volume of the reagent to 10 times its volume and use 5 cc. of this diluted reagent. It must be freshly diluted daily.

Transfer 0.5–15 cc. of sample solution containing 0.01–0.3 mg. of phosphorus pentoxide to a 50 cc. volumetric flask having a mark at 30 cc. In another flask take an equal amount of standard. Add reagents to the standard similar to those known to be present in the sample. To each add 5 drops of a saturated aqueous solution of  $\alpha$ -dinitrophenol (2,4-dinitrophenol) and neutralize to a very faint yellow with 2 per cent sodium bicarbonate or *N* sulfuric acid. To each add 5 cc. of *N* sulfuric acid and mix. To each add 5 cc. of 8 per cent sodium bisulfite solution. Dilute to about 30 cc. and mix well. Let stand over night or heat on a steam bath for 1 hour. Add 5 cc. of the diluted reagent equivalent to 0.5 cc. of the concentrated reagent and heat on the steam bath for 30 minutes.

The full color develops in 3 days at 20–30°, 10 hours at 50°, 3 hours at 70° or 30 minutes at 95–100°. By direct boiling it develops in 4 to 5 minutes. Let cool to room temperature and dilute to 50 cc. with distilled water. Mix well and compare sample and standard by balancing. The color is stable for 2 to 3 days in the dark in stoppered flasks. No blue color should appear in a slightly yellowish phosphorus-free blank. Standard and sample must receive identical treatment as the color is a function of pH. The color cannot be diluted. If the color of the sample is too dark repeat with a smaller sample. Sample and standard must not differ by more than 30 per cent. The color will not be altered by 2 mg. of ferric oxide nor 10 mg. of nitrate.

*Reduction with Benzidine.*<sup>79</sup> To the sample and standard add 4 drops of a solution of ammonium citrate containing 50 grams of citric acid and 63 cc. of 25 per cent ammonium hydroxide per 100 cc. Prepare the reagent by dissolving 15 grams of ammonium molybdate in 100 cc. of water and slowly pouring this into 100 cc. of 1:2 nitric acid. Add 5 drops of this reagent and mix. Add 4 drops of a solution of 0.25 gram of benzidine hydrochloride in 5 cc. of 1:2 acetic acid, diluted to 100 cc. Mix and compare the standard and sample. The method was developed for mineral analysis.

**Standard Phosphate Solution**—Prepare the following standard so-

lutions for use in the various determinations. Dry monopotassium phosphate in a desiccator over sulfuric acid for several days. Weigh 0.4394 gram, dissolve in water, add 10 cc. of 1:3 sulfuric acid and dilute to 1 liter. This standard keeps indefinitely. It contains 0.1 mg. of phosphorus per cc. Its equivalent in terms of phosphorus pentoxide is 0.229 mg. or in terms of phosphoric acid 0.317 mg.

Prepare a more dilute standard by dilution of 10 cc. of the above to 100 cc. This contains 0.01 mg. of phosphorus per cc. The corresponding equivalents are 0.0229 mg. of phosphorus pentoxide or 0.0317 mg. of phosphoric acid. To each diluted standard add 10 cc. of 1:3 sulfuric acid during dilution if it is to be kept for any length of time.

**Permanent Standards**—The quality of color of permanent glass standards for use with the Hellige comparator matches the solutions.<sup>112</sup> They must be standardized for the special procedure in use in any laboratory. It is possible to interpolate 10 degrees of color between the successive standards. The accuracy obtainable is sufficient for ordinary purposes.

Reduction of 2.5 grams of ammonium molybdate in 100 cc. of 10 *N* sulfuric acid with stannous chloride gives a blue which may be diluted with 10 *N* sulfuric acid for permanent standards.<sup>131</sup> The Pulfrich step photometer has also been used for reading the colors of the resulting solutions instead of using a natural standard.<sup>132</sup>

#### PHOSPHATES IN WATER BY STANNOUS CHLORIDE REDUCTION OF THE MOLYBDATE

The method which follows<sup>133</sup> is sufficiently different from the established procedures to indicate the desirability of separate presentation. It is an improvement on an earlier procedure.<sup>105</sup>

Color develops immediately and fading is very slow. Organic matter and silica up to 3500 p.p.m. in the water do not interfere. Temperature is not an important factor. Ferrous iron, aluminum or copper up to 50 times the phosphate content may be present. Ferric iron in excess of 6 p.p.m. causes a greenish shade. Carbonates or sulfates up to 400 times the phosphate content do not alter the color, nor does a substantial nitrate

---

<sup>131</sup> A. H. Meyer, *Science* 72, 174 (1930).

<sup>132</sup> Carl Urbach, *Biochem. Z.* 239, 28-34 (1931).

<sup>133</sup> Jason E. Faber and Guy E. Youngburg, *Ind. Eng. Chem., Anal. Ed.* 4, 107-9 (1932).



content. The method will detect 0.01 p.p.m. of phosphate and at 0.5–48 p.p.m. is accurate within 3 per cent, results being low.

**Reagents—Molybdate Reagent.** Dissolve 37.5 grams of sodium molybdate, or 64 grams of ammonium molybdate, in water and dilute to 500 cc. Prepare 10 *N* sulfuric acid by mixing 450 cc. of concentrated acid with 1156 cc. of water. For greater accuracy, titrate this acid solution. Mix 500 cc. of the molybdate solution with 500 cc. of 10 *N* sulfuric acid.

**Stannous Chloride Reagent.** Dissolve 10 grams of crystallized stannous chloride in 25 cc. of concentrated hydrochloric acid as a stock solution. As reagent, dilute 1 cc. of the stock solution to 200 cc. Discard when turbidity appears.

**Procedure—**Place 35 cc. of sample water in a test tube, calibrated at 35 cc. and 50 cc. To similar tubes add 1 cc. and 10 cc. of standard phosphate solution. If desired for greater accuracy, insert intermediate quantities. Dilute the standards to 35 cc. with distilled water. To each of the tubes add 10 cc. of the molybdate reagent. Mix and add 5 cc. of the stannous chloride reagent. Mix and compare.

**Standards—**As a stock solution, dissolve 2.506 grams of anhydrous monopotassium phosphate in water and dilute to 100 cc. For analysis of natural waters, dilute 2 cc. of this to 1 liter. Each cc. further diluted to 35 cc. as in the procedure is equivalent to 1 p.p.m. of phosphate radical,  $\text{PO}_4^{=}$ . For analysis of boiler waters, dilute 10 cc. to 1 liter. Each cc. of this diluted to 35 cc. is equivalent to 5 p.p.m. of phosphate radical.

#### PHOSPHATES IN SEA WATER BY STANNOUS CHLORIDE REDUCTION OF THE MOLYBDATE

While superficially closely related to other estimations of phosphate the amounts of foreign salts necessarily present in sea water radically affect this estimation. The procedure<sup>134a</sup> is therefore given separately. Various modifications<sup>134b,c</sup> have been applied.

The color developed is affected by acidity.<sup>86</sup> It has been applied

<sup>134a</sup> Rex J. Robinson and Henry E. Wirth, *Ind. Eng. Chem., Anal. Ed.* 7, 147-50 (1935).

<sup>134b</sup> D. Florentin, *Ann. chim. anal. chim. appl.* 3, 295-6 (1921).

<sup>134c</sup> W. R. G. Atkins, *J. Marine Biol. Assoc. United Kingdom* 13, 119-50 (1923).

spectrophotometrically <sup>134d</sup> and photometrically. <sup>134e,f</sup> Detailed investigation has indicated the desirability of the Truog and Meyer <sup>86</sup> or Atkins <sup>134c</sup> procedures with some slight modification. The major change is reduction of the amount of stannous chloride to avoid greenish colors, even though this reduces the intensity of the color developed.

**Procedure**—Transfer 50 cc. of sample to a tube. Add 1 cc. of a molybdate reagent prepared by dissolving 25 grams of ammonium molybdate in 250 cc. of distilled water and adding to 750 cc. of sulfuric acid containing 375 cc. of concentrated arsenic-free sulfuric acid. Mix and add 1 drop of a stannous chloride reagent prepared by dissolving 25 grams of stannous chloride dihydrate in 100 cc. of concentrated hydrochloric acid, diluting to 1 liter with water, and stored under a protective layer of oil.

Read photometrically and compare with previously developed curves or read against sea water containing a known amount of phosphorus. Alternatively compare with a standard developed in distilled water and multiply the result obtained by 1.15, since the intensity developed in a sea water standard is not as great as in a distilled water standard.

#### PHOSPHATE AS SILVER PHOSPHATE

By preparation under suitably standardized conditions, a colloidal dispersion of silver phosphate can be obtained suitable for nephelometric comparison. <sup>135</sup>

**Sample**—Prepare as for the reduction method and compare with standards as quoted there. <sup>135a</sup>

**Procedure**—Make the solution slightly alkaline to phenolphthalein by careful addition of 20 per cent sodium hydroxide solution, noting the amount used. Make just acid with approximately 0.5 *N* sulfuric acid and cool. Make neutral with approximately 0.1 *N* sodium hydroxide solution. To the neutralized solution add 1 cc. of 10 per cent ammonium

h, *Con. perm. intern. pour l'exploration de la mer, Rapports et proces verbaux* 53, 36 (1929).

<sup>134e</sup> M. Giani, *Giorn. chim. ind. applicata* 15, 432-5 (1933).

<sup>134f</sup> C. Urbach, *Mikrochemie* 13, 31-54 (1933).

<sup>135</sup> W. R. Bloor, *J. Biol. Chem.* 22, 133 (1915); *Ibid.* 24, 447 (1916).

<sup>135a</sup> See pp. 486-96, 507-8.

sulfate solution and 1.5 cc. of 0.1 *N* sodium hydroxide solution. Dilute to such a volume that the phosphorus content is about 0.005 mg. per cc.

To the standard phosphate add a drop of phenolphthalein solution and the amount of sodium hydroxide solution required to neutralize the sample. Bring to neutrality with concentrated sulfuric acid and complete the neutralization in the same way as with the sample, making the final volume the same as that of the sample.

Place 10 cc. of neutralized 2 per cent silver nitrate solution in each of two 25 cc. volumetric flasks. Add 10 cc. of standard and sample through funnels which have been drawn down to deliver 10 cc. in about 15 seconds. Gently rotate the flasks while the solution is being added. Rinse out the original containers with distilled water and pour the wash-water through the funnels. Rinse the funnels with distilled water, make up to volume and read in the nephelometer. Chlorides must be rigidly excluded from all reagents used. Acid ashing is necessary to remove chlorides present in the original sample.

#### PHOSPHATE BY THE STRYCHNINE REAGENT

The reagent originally devised by Pouget and Chouchak<sup>136</sup> has been modified by Kober and Egerer<sup>137</sup> and by Bloor<sup>138,138a</sup> for nephelometric comparison. The turbidity is suitable for comparison with an artificial standard using a calibration curve.<sup>139</sup>

**Sample**—Prepare as for the reduction methods<sup>140</sup> and compare with the quantity of standard specified there.

**Reagent**—A solution of sodium molybdate is first prepared. Mix 72 grams of pure molybdic anhydride with 300 cc. of water and neutralize with 40 per cent sodium hydroxide solution. If the anhydride is pure this will require 100 cc. The reagents must be free from all but traces of phosphorus. Boil for one-half hour, adding water to keep the volume constant and more alkali if the solution becomes turbid. The latter condition would be due to boiling off traces of ammonia present in the an-

I. Pouget and D. Chouchak, *Bull. soc. chim.* [3], 5, 104 (1909); *Ibid.* [3], 9, 649 (1911).

<sup>137</sup> P. A. Kober and G. Egerer, *J. Am. Chem. Soc.* 37, 2373 (1915).

<sup>138</sup> W. R. Bloor, *J. Biol. Chem.* 36, 33 (1918).

<sup>138a</sup> Cf. Carlo Crema, *Diagnostica tec. lab. (Napoli)* Riv. mensile; 1, 740-3 (1930).

<sup>139</sup> J. Avellar de Loureiro, *Biochem. Z.* 224, 337-46 (1930).

<sup>140</sup> See pp. 486-96, 507-8.

hydride. Add 1 gram of powdered talc and let stand for 5 minutes. Filter and wash the filter once, adding the washings to the filtrate. This will contain about 100 grams of sodium molybdate. Dilute to a known volume.

To a portion of the above solution containing 30-35 grams of sodium molybdate, or an equivalent amount of solution of sodium molybdate in distilled water, add 250 cc. of 1:1 hydrochloric acid, stirring well. The precipitate first formed dissolves in the excess of acid. Follow this with 500 cc. of water, 40-50 cc. of saturated strychnine sulfate solution, 200 cc. more of 1:1 acid and 500 cc. more of water, stirring each in turn. Let stand over night and decant the clear upper layer. Filter the balance through hardened paper known to be free from phosphorus. The solution should be perfectly clear and practically colorless and is now ready for use.

A simpler method of preparation<sup>141</sup> is to dissolve 6 grams of sodium carbonate in 140 cc. of water and dissolve 19 grams of molybdic anhydride in this by heating. To this add 50 cc. of concentrated hydrochloric acid and 20 cc. of 2 per cent strychnine sulfate solution. The reagent so prepared is stable for several weeks.

**Procedure**—If the ashing method has introduced acids which were later neutralized, or salts, the equivalent must be added to the standard. Nephelometric methods are more susceptible to such variations between standard and sample than are colorimetric methods.

A typical case will best illustrate this. For determination of total phosphate in blood a sample has been ashed by the nitric-sulfuric acid method and diluted with water. It is neutralized to phenolphthalein with 10 per cent sodium hydroxide solution, noting the volume used. The same amount is added to the standard. The sample is made acid with a drop of 1:3 sulfuric acid and the standard titrated to faint acidity with the same acid. The result, if nitric acid has been efficiently removed in ashing, is a sample and standard each containing the same amount of sodium sulfate. They are then made to the same volume which should be such as to make the concentration of phosphorus about 0.005 mg. per cc. The final solution for determination must be faintly acid.

Prepare two 50 cc. volumetric flasks, each containing 25 cc. of the strychnine reagent. To one add 5 cc. of sample and to the other the same volume of standard, keeping the flask rotating during the additions. Mix

---

<sup>141</sup> Luigi Belladen, Ugo Scazzola and Renato Scazzola, *Ann. chim. applicata* 23, 517-21 (1931).

well, let stand at least 3 minutes, dilute to volume with distilled water, mix and compare in a nephelometer. If standard and sample do not differ by more than 25 per cent there is no significant change in 20 minutes.

Other investigators<sup>142</sup> use 2 cc. of reagent, let stand for 30 minutes and then add 2 cc. of 10 per cent gum arabic solution. Comparison may then be made any time within two and one-half hours. Another<sup>143</sup> carries out the determination in 2 *N* sulfuric acid.

#### PHOSPHORUS IN CAST IRON BY THE STRYCHNINE REAGENT

This determination according to Kober<sup>144</sup> may be made nephelometrically with the strychnine reagent.

**Procedure**—Dissolve 2 grams of iron borings in 100 cc. of 1:2 nitric acid by boiling, cool under the tap and dilute to 100 cc. Pipet a 5 cc. sample and boil for 2 minutes with 5 cc. of concentrated sulfuric acid, taking care not to cause any considerable evolution of sulfur trioxide fumes. Dilute to 100 cc. and filter. Dilute 25 cc. of filtrate to 100 cc. Pipet 10 cc., add 35 cc. of 0.5 *N* hydrochloric acid and treat with 5 cc. of the reagent. Compare with a standard produced from an iron of known phosphorus content.

#### PHOSPHATE BY THE STRYCHNINE REAGENT AS DEVELOPED WITH FERROCYANIDE

The method as developed for serum<sup>145</sup> has been applied to many other materials, such as saliva, with or without ashing.<sup>125</sup> Accuracy within 5 per cent is obtained.

**Sample**—*Serum*. Add 5 cc. of 6 per cent trichloroacetic acid to 1 cc. of serum in a centrifuge tube. Mix well and let stand for 4 minutes. Centrifuge and transfer 5 cc. of the supernatant liquid to a 15 cc. tube.

**Reagent**—Dissolve 50 grams of ammonium molybdate in 150 cc. of water. Pour this into 3 volumes of 1:2 nitric acid. To 3 volumes of the acid solution add 1 volume of a 1.5 per cent solution of strychnine

<sup>142</sup> Ludwig Pincussen and Franz Jalusburger, *Biochem. Z.* 177, 140-3 (1926).

<sup>143</sup> Hans Kleinmann, *Biochem. Z.* 174, 43-52 (1926).

<sup>144</sup> P. A. Kober, *J. Ind. Eng. Chem.* 10, 556 (1918).

<sup>145</sup> F. S. Tisdale, *J. Biol. Chem.* 50, 329-37 (1922).

sulfate in water. Let stand for 1 to 2 days and filter. The reagent will keep at least a month.

**Procedure**—Add 1 cc. of water and 2 cc. of the molybdate reagent drop by drop with shaking. Let stand with occasional shaking for 10 minutes and centrifuge at 1500 r.p.m. for 3 minutes. Decant, dry the mouth of the tube and add 3 cc. of water. Wash the precipitate with this, centrifuge and decant. Repeat the washing operation.

Dissolve the precipitate in 2 cc. of 1 per cent sodium hydroxide solution and wash into a 100 cc. flask with 28 cc. of water. Add 20 cc. of 20 per cent potassium ferrocyanide solution and 10 cc. of concentrated hydrochloric acid. Mix and let stand for 10 minutes. Dilute to volume and mix. Compare with a standard similarly prepared containing 0.05 mg. of phosphorus. Approximate adjustment of the relative volumes of sample and standard can be made by comparison of the volumes of centrifuged precipitate.

#### PHOSPHATE BY THE QUININE REAGENT

The yellow color produced by the reaction between phosphate and a special molybdate-quinine reagent may be used for colorimetric estimation.<sup>147</sup>

**Sample**—Prepare according to previous methods, noting that a trace of silica is permissible but iron must be absent. Iron may be removed with cupferron. A suitable sample contains 0.002–0.02 mg. of phosphorus pentoxide.

**Reagent**—Dissolve 1 gram of quinine sulfate in 50 cc. of 1:5 nitric acid. Add a saturated solution of barium hydroxide until no further precipitation occurs. Filter and mix the filtrate with 40 grams of ammonium molybdate dissolved in 500 cc. of 1:1 nitric acid. Dilute to 1 liter and mix well.

**Procedure**—Dilute the sample and a series of standards to about 45 cc. each, in Nessler tubes. To each add 2 cc. of 1:3 nitric acid and 2 cc. of reagent. Dilute each to 50 cc. and compare.

#### PHOSPHATE BY URANIUM ACETATE AND POTASSIUM FERROCYANIDE

This indirect method, originally<sup>148</sup> found to give high results, has

<sup>147</sup> A. Gregoire, *Bull. soc. chim. Belg.* 29, 253 (1920)

<sup>148</sup> R. B. Gibson and C. Estes, *J. Biol. Chem.* 6, 349 (1909).

been further developed. The phosphate is precipitated with uranium acetate and filtered. This may be redissolved and the uranium estimated with potassium ferrocyanide.<sup>149</sup> The amount of uranium precipitated may also be estimated from that remaining in solution and the phosphorus calculated from the amount precipitated.<sup>150</sup>

**Sample—General.** This must be made protein-free by the usual procedures.

**Lipoid Phosphorus.**<sup>151</sup> Extract the lipoids from blood with a mixture of 3 parts of ether and 1 part of alcohol. Evaporate this solution and destroy organic matter by acid ashing.<sup>152</sup> Dissolve the ash in 2 cc. of 0.05 N sulfuric acid.

**Procedure—**To 2 cc. of sample add 2 cc. of 0.01 N uranium acetate solution. This is 2.1215 grams per liter. Prepare a solution of 105 grams of ammonium acetate and 100 cc. of glacial acetic acid per liter. Add 1 cc. of this mixture and dilute to 10 cc. with water. Centrifuge to settle the precipitate.

**Direct Estimation.** Decant and wash the precipitate twice with 5 cc. of water to which 0.5 cc. of the acetate buffer has been added. Dissolve the precipitate in 5 cc. of 5 per cent trichloroacetic acid, add to 2 cc. of 0.5 per cent potassium ferrocyanide solution and compare with a standard amount of phosphate similarly treated.

**Estimation of Excess Uranium.** Pipet out 5 cc. of the clear supernatant liquid. Add this to 2 cc. of 0.5 per cent potassium ferrocyanide solution. Prepare a blank which has been similarly treated but in which the sample has been substituted by distilled water. Compare the two after 30 minutes. Calculate the amount of uranium acetate precipitated and multiply by 0.073 to give the result in terms of phosphorus, 0.167 in terms of phosphorus pentoxide or 0.224 in terms of phosphate radical.

#### PHOSPHORUS IN COMBUSTIBLE GASES

Mix the gas with oxygen and ignite by an electric discharge.<sup>153</sup> Collect the water formed and determine phosphorus as phosphate by the usual methods.

S. L. Leiboff, *J. Biol. Chem.* 79, 611-19 (1928).

Hinsberg and K. Lang, *Biochem. Z.* 196, 465-70 (1928).

S. L. Leiboff, *J. Biol. Chem.* 80, 211-4 (1928).

<sup>152</sup> See pp. 497-8.

<sup>153</sup> Witold Hennel, *Przemysl Chem.* 11, 634-7 (1927).

## PHOSPHORUS BY SILVER NITRATE

The amount of elementary phosphorus in oil solution can be estimated from the brown color produced with silver nitrate dissolved in acetone,<sup>154,155</sup> The color is completely developed in 15 minutes. A trace of water promotes deflocculation. The standards, properly sealed, are stable.

**Procedure**—Dilute 1 cc. of sample to 10 cc. with a mixture of 40 cc. of ether, 20 cc. of alcohol and 5 cc. of acetone. Add 0.2 cc. of a solution of 0.25 gram of silver nitrate in 100 cc. of acetone.

To 9 cc. of the solvent mixture add 0.2 cc. of the silver nitrate reagent and sufficient of a standard oil containing 0.1 mg. of phosphorus per cc. to give approximately the same color as that developed in the sample. This is used as an approximation. Dilute the sample with oil, free from phosphorus, until it comes within the range of a series of standards containing 0.05–0.1 mg. of phosphorus per gram of oil. Repetition of the procedure on the properly diluted sample then gives the true value of the sample.

---

<sup>154</sup> C. Stick, *Z. angew. Chem.* 40, 1014 (1927).

<sup>155</sup> Helga Wael, *Dansk. Tids. Farm.* 4, 197-208 (1930).



## CHAPTER L

### SILICA

#### SILICA AS THE SILICOMOLYBDATE

AMMONIUM silicomolybdate in acid solution has an intense yellow color,<sup>1,2</sup> stated to be  $\text{H}_8[\text{Si}(\text{MoO}_7)_6]\text{H}_2\text{O}$ . This property is used for estimation of silica in amounts under 60 mg. per liter, such as are found in water. It has been stated that phosphates, arsenates and arsenites in moderate amounts give no color<sup>3</sup> and that as much as 20 mg. of iron per liter will not interfere. These statements result from a curious combination of compensating errors.

Silica gives correct results with phosphate absent, or with an amount present equal to 100 mg. of phosphorus pentoxide per liter. With lesser amounts of phosphorus results are too high and with larger amounts they are too low.<sup>4,5,6</sup> This is partly due to a change in the compound formed, between the two possible types formed in acid solution,  $3\text{R}_2\text{O} \cdot \text{P}_2\text{O}_5 \cdot 18\text{MoO}_3$  and  $3\text{R}_2\text{O} \cdot \text{P}_2\text{O}_5 \cdot 24\text{MoO}_3$ .<sup>7</sup> Others report that with sufficient excess of phosphoric acid present the phosphate does not affect the color and destroys interference due to iron.<sup>7a</sup> Its addition has been recommended in estimation of silica in iron.<sup>7b</sup> Other sources of error are precipitation of ammonium phosphomolybdate at high phosphate concentrations and variation in the acidity of the solution. Higher acidities cause a deeper color. Extraction of silica from the glassware used is avoided by prior treatment with hot dilute alkali and checked by running a blank with a known amount of silica.<sup>8</sup>

<sup>1</sup> A. Jolles and F. Neurath, *Z. angew. Chem.* 11, 315-6 (1898).

<sup>2</sup> F. Dienert and F. Wandenbuleke, *Compt. rend.* 176, 1478 (1923).

<sup>3</sup> W. R. G. Atkins and E. G. Wilson, *Biochem. J.* 20, 1223 (1926); *J. Marine Biol. Asscn. United Kingdom* 14, 89 (1926); *Ibid.* 15, 191 (1928); *Ibid.* 16, 822 (1930).

<sup>4</sup> Lewis A. Thayer, *Ind. Eng. Chem., Anal. Ed.* 2, 276-83 (1930).

<sup>5</sup> Earl J. King, *Ind. Eng. Chem., Anal. Ed.* 3, 117-8 (1931).

<sup>6</sup> Lewis A. Thayer, *Ind. Eng. Chem., Anal. Ed.* 3, 118 (1931).

<sup>7</sup> Arnfeld, Dissertation, Berlin, pp. 1-60 (1899).

<sup>7a</sup> I. P. Alimarin and V. S. Zverev, *Trans. Inst. Econ. Mineral* (U. S. S. R.) No. 63, 15 pp. (1934).

<sup>7b</sup> Hans Pinsl, *Giesserei* 22, 78-9 (1935).

<sup>8</sup> Otto Liebknecht, Lothar Gerb and Erich Bauer, *Z. angew. Chem.* 44, 860-3 (1931).

Neither ferrous nor ferric iron in the absence of phosphate causes serious interference up to a concentration of 20 mg. per liter. In the presence of phosphate they do. Iron and phosphorus must therefore be removed. Phosphate may be precipitated by magnesia mixture.<sup>9</sup> A convenient procedure is to precipitate iron as ferric phosphate and remove excess phosphate as calcium or magnesium phosphate. Excess of calcium or magnesium ions does not interfere.

**Sample—Sulfites Present.** Oxidize with permanganate solution.

**Colloidal Silica Present.** If some of the silica is colloidal, this must be dissolved. To do this, add 0.2 gram of sodium bicarbonate to 50 cc. of sample solution in a platinum dish. Heat on a boiling water bath an hour, then add 2.4 cc. of *N* sulfuric acid. This neutralizes the bicarbonate and leaves the silica in solution. Cool and dilute to 50 cc.

**Removal of Iron and Phosphates.**<sup>4,8,12</sup> When it is desired to remove iron and phosphates from a sample, proceed as follows: To 50–100 cc. of sample solution add 3 cc. of a 9.3 per cent solution of hydrated disodium phosphate, 3 cc. of a 20 per cent solution of calcium chloride hexahydrate and 1 gram of finely powdered calcium carbonate. Heat for 10 minutes on a water bath with frequent shaking. Filter while hot and wash the precipitate once with hot water. Dilute to a known volume and use a suitable aliquot as sample.

**Boiler Water.**<sup>12a</sup> Prepare a buffer solution by mixing 6 parts of a solution of 12.40 grams of boric acid and 100 cc. of *N* sodium hydroxide per liter, with 4 parts of 0.1 *N* sodium hydroxide solution.

Mix 110 cc. of sample with 50 cc. of this buffer solution. Add 2 cc. of *M* calcium chloride solution and mix vigorously. A flocculent precipitate of calcium phosphate settles rapidly. Let stand at least 1 hour and preferably 2 hours with occasional stirring, and filter. Use 50 cc. of the filtrate as sample. Subtract a blank on the buffer from the result obtained and multiply the result by 1.54 to correct for dilution by the buffer.

**Procedure—Soluble Silica.** Add 2 cc. of 10 per cent ammonium molybdate solution to the treated sample and adjust to a pH value of about 1.5. This should require 8 drops of 10 per cent acetic acid. Shake

<sup>9</sup> Earl J. King, *J. Biol. Chem.* 80, 25-31 (1928); *Bull. soc. chim. biol.* 12, 903-9 (1930).

<sup>12</sup> Cf. A. Jolles, *Z. angew. Chem.* 45, 150 (1932).

<sup>12a</sup> M. C. Schwartz, *Ind. Eng. Chem., Anal. Ed.* 6, 364-7 (1935).

and compare with the standard after 10 minutes. The use of 4 drops of concentrated sulfuric acid per 50 cc. of sample has been recommended for use with sea water<sup>13</sup> and 1 cc. of 3:2 hydrochloric acid with boiler water containing buffer.

*Colloidal Silica.* To determine colloidal silica run two samples, in one of which the procedure with sodium bicarbonate is used to put the total silica in true solution, the other not so treated. The difference between the two results is colloidal silica. This method has been used for determination of that portion of sodium silicate in true solution as silicate ion.

**Standard—From Silica.**<sup>14</sup> Dissolve about 5 grams of precipitated and washed silica in excess sodium hydroxide solution, prepared by the action of metallic sodium on water in a platinum dish. Dilute to a large volume and make faintly acid with nitric acid. Dilute to a definite volume and determine silica gravimetrically in an aliquot. Dilute so that 1 cc. corresponds to 1 mg. of silicon dioxide.

*From Sodium Silicate.* Weigh a sample of analyzed sodium silicate solution to contain 1 gram of silica. Dissolve in about 500 cc. of water, add nitric acid until faintly acid and dilute to 1 liter. Each cc. will then contain 1 mg. of silica. This must be treated with sodium bicarbonate before use as some of the silica will be colloidal.

**Artificial Standard—Picric Acid.** The use of 25.6 mg. of vacuum-dried picric acid per liter has been recommended as standard instead of 50 mg. of silica per liter.<sup>8,13,15-17,18a</sup> Its equivalence will be altered by modifications of the procedure, and it should be checked against any specific procedure with which it is to be used. The usual objections to artificial standards apply but the transmittance curves are not sufficiently different from those of the natural standard to preclude its use.<sup>21a</sup> Fungus growth has been found to have no effect in lowering the color of picric acid standards.<sup>13</sup> While exposure to dust will cause reduction in color, no such effect occurs in properly protected standards.<sup>3</sup>

<sup>13</sup> Thomas G. Thompson and Harold G. Houlton, *Ind. Eng. Chem., Anal. Ed.* 5, 417-8 (1933).

<sup>14</sup> A. G. Woodman and L. L. Cayvan, *J. Am. Chem. Soc.* 23, 96-107 (1901).

<sup>15</sup> Earl J. King and C. C. Lucas, *J. Am. Chem. Soc.* 50, 2395-7 (1928).

<sup>16</sup> F. Dienert and F. Wandenbulke, *Bull. soc. chim.* 33, 1131-90 (1923).

<sup>17</sup> Earl J. King, *Contrib. Can. Biol. Fisheries* 7, Nos. 8-11, Series D, Nos. 1-4, 121-5 (1931).

<sup>18a</sup> Cf. Thresh and Beale, "Examination of Waters and Water Supplies," 3rd ed., p. 347. Churchill, London (1925).

<sup>21a</sup> H. W. Swank and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 6, 348-50 (1934).

*Potassium Chromate.* A solution of 0.63 gram of potassium chromate<sup>22</sup> or the equivalent amount of potassium bichromate per liter in a solution buffered to above pH 8 by addition of 0.5 per cent of sodium tetraborate<sup>21a</sup> is equivalent to 100 mg. of silica per liter. The reddish yellow nature of the color<sup>21b</sup> does not then interfere. This solution without buffer is the standard of the American Public Health Association. With the buffer it is more dependable.

#### SILICA AND PHOSPHORUS BY AMMONIUM MOLYBDATE

Methods have been developed by which phosphorus and silica are determined, the phosphorus estimated alone and silica obtained by suitable calculation of the difference.<sup>14,24-26</sup> The color caused by silica differs from that caused by phosphorus in that it reaches its full intensity only after some hours, whereas phosphorus shows its full color after 20 minutes. Another property of the silica color, on which this method is based, is that its intensity at the end of 1 hour and 20 minutes is twice that at the end of 20 minutes.

**Sample**—Treat the sample as in the preceding method.<sup>27</sup> Prepare two samples of the same solution to be examined at the same time. A standard is used which contains only phosphorus.<sup>28</sup> Take 50 cc. portions of the sample solution and a suitable volume of standard diluted to 50 cc.

**Procedure**—*Phosphorus.* Acidify 1 sample and the standard with 1 cc. of concentrated nitric acid, add 2 cc. of 10 per cent ammonium molybdate solution and allow to stand for 20 minutes. Compare. At the time these are acidified and the ammonium molybdate solution added, add a similar amount of ammonium molybdate solution but no nitric acid to the other sample. After 1 hour has elapsed acidify this second sample with 1 cc. of concentrated nitric acid and allow to stand for 20 minutes. Compare with the same standard as was used with the other sample. The reading of the first sample is called the A reading and the reading of the second sample the B reading.

<sup>21b</sup> W. Steffens, *Chem.-Ztg.* 54, 996-7 (1930).

<sup>22</sup> L. W. Winkler, *Z. anorg. Chem.* 27, 511-2 (1914).

<sup>24</sup> F. P. Veitch, *J. Am. Chem. Soc.* 25, 169-84 (1903).

<sup>25</sup> O. Schreiner, *Ibid.* 25, 1056-62 (1903).

<sup>26</sup> A. T. Lincoln and Perry Barker, *Ibid.* 26, 975-80 (1904).

<sup>27</sup> See p. 518.

<sup>28</sup> See p. 522.

The silica reading at the end of 1 hour and 20 minutes from the time the ammonium molybdate solution was added and at the end of 20 minutes from the time the nitric acid was added to it, represents the same amount of phosphorus as the reading at the end of 20 minutes by the first sample and in addition a reading for silica twice that given at the end of 20 minutes by the first sample. If the B reading is twice the A reading there is no phosphorus present. If the B reading is less than twice the A reading, subtract the difference between them from the A reading to obtain the reading for phosphorus. Since phosphorus is determined by difference the error in estimation of small amounts will be serious. In many cases therefore, to estimate small amounts of phosphorus a measured amount is added, and then subtracted from the result.

*Silica.* Phosphorus having been estimated, determine silica by the same method, making the comparison against a standard containing only silica.<sup>29</sup> This silica determination is then corrected for the phosphorus previously determined. This silica standard may be prepared at the same time as the original samples and the same sample used as for phosphorus. The first sample may be kept for 4 to 5 hours and compared against a standard prepared simultaneously and kept for the same length of time. The method is by dilution only.

#### PHOSPHATE AND SILICA TOGETHER AS THE MOLYBDATE BY HYDROQUINONE REDUCTION

A modification of the well known hydroquinone reduction of Briggs,<sup>30</sup> and Bell and Doisy<sup>31</sup> has been recommended<sup>32</sup> for estimation of phosphorus and silica together expressed as silica, after which, by estimation of total phosphorus alone, silica is obtained by difference. For convenience, methods for inorganic and organic phosphorus have been repeated in part.

**Sample—Sugar for Phosphorus and Silica.** Mix 5 grams of sugar with 0.2 gram of sodium and potassium carbonates in a platinum dish, freshly cleaned by carbonate fusion. Carefully char to a white ash. Incinerate at 550° in an electric muffle and finally fuse over a free flame to a clear melt. Let cool and dissolve in 15 cc. of cold distilled water. Add

<sup>29</sup> See p. 519.

<sup>30</sup> A. P. Briggs, *J. Biol. Chem.* 53, 13 (1922); *Ibid.* 59, 255 (1924).

<sup>31</sup> R. D. Bell and E. A. Doisy, *J. Biol. Chem.* 44, 55 (1920).

<sup>32</sup> S. Byall and J. A. Ambler, *Ind. Eng. Chem., Anal. Ed.* 4, 325-7 (1932).

1 cc. of 1:4 acetic acid to neutralize carbonates and transfer to a tube without filtering.

*Sugar for Total Phosphorus.* Follow instructions previously given.<sup>33</sup>

*Inorganic Phosphorus.* Use a solution of suitable concentration.

**Procedure**—Add to the sample 5 cc. of an ammonium molybdate solution made by dissolving 25 grams in 300 cc. of water and then mixing with 200 cc. of water containing 75 cc. of concentrated sulfuric acid. Mix and add 1 cc. of a freshly prepared 20 per cent sodium sulfite solution. Mix and add 1 cc. of a solution of 0.5 gram of hydroquinone in 100 cc. of water containing 1 drop of concentrated sulfuric acid to retard oxidation of the reagent. Mix and dilute to 100 cc. Mix and let stand for 30 minutes. Compare with standards similarly prepared from 0.1 to 4.0 cc. of the standard phosphate solution.

**Standard**—Dissolve 0.4394 gram of monopotassium phosphate in water and dilute to 1 liter. Dilute 25 cc. of this stock solution to 200 cc. to give a solution containing 0.0287 mg. of phosphorus pentoxide per cc. Each cc. is equivalent to 0.0363 mg. of silica.

**Calculations**—The results are for total phosphorus, inorganic phosphorus, or for phosphorus and silica, according to the preparation of sample.<sup>1</sup> For convenience calculations are carried through in terms of cc. of standard solution, then multiplied by suitable factors.

*Silica.* Subtract the total phosphorus from the phosphorus and silica and multiply by 0.0363 to get the amount of silica in mg. For parts per million multiply by 200.

*Organic Phosphorus.* Subtract half the value for inorganic phosphorus from that for total phosphorus and multiply by 0.0287. For parts per million multiply by 200.

*Inorganic Phosphorus.* Multiply the corrected value for inorganic phosphorus by 0.0287. For parts per million multiply by 100.

#### SILICA BY REDUCTION OF THE SILICOMOLYBDATE

When ammonium molybdate is added to a silicate or silicic acid solution a silicomolybdate is formed. By addition of a reducing agent such as sodium sulfite the silicomolybdate is reduced to molybdenum blue. At

<sup>33</sup> See p. 493.

a low acidity excess reagent and phosphomolybdates are unaffected.<sup>35</sup> Bertrand<sup>36</sup> has criticized the method but Foulger<sup>37</sup> has shown that phosphate free from silica gives no color with the reagent in solutions having the degree of acidity recommended by Isaacs. With a sufficient concentration of phosphate the tendency is to retard the reduction of the silicomolybdate. This may be offset by a slight increase of acidity. Good evidence<sup>38</sup> has been presented to show that arsenic and phosphorus behave almost identically. It was also shown that stannous chloride is the best reducing agent for phosphomolybdate and arsenomolybdate. Oberhauser and Schormüller<sup>39</sup> believe that it is also the best for the silicomolybdate. They use a freshly prepared, strongly alkaline stannite solution, adding only a small amount. Hydroxylamine followed by sodium hyposulfite is also suitable for use as reducing agent.<sup>40</sup> Reduction with hydroquinone to determine both phosphorus and silica has also been recommended, with a somewhat different procedure, for use in sugar analysis.<sup>32</sup> By suitable adjustments of the composition of the sample it is probable that nearly all procedures applicable to phosphomolybdate reduction could also be applied to silicomolybdate reduction.

Iron must be removed or a special standard used containing the same amount of iron. A blank must be run on the reagents as they are apt to dissolve silica from the bottles in which they are kept.

**Sample—Tissue.**<sup>35</sup> Weigh 0.5 gram of sample into a platinum crucible. Add 1 cc. of a saturated solution of boric acid, 1 cc. of a 5 per cent solution of calcium nitrate and 2 cc. of concentrated nitric acid. Heat on a water bath until dissolved. Heat over a free flame until the residue begins to char. Add concentrated nitric acid and heat again until a white ash is obtained. Moisten with 0.5 cc. of concentrated nitric acid and heat to drive off excess acid. Add 3 cc. of water and 3 cc. of a 4 per cent sodium hydroxide solution. This sample was designed for development of color by sodium sulfite.

**Tissue. Alternative Method.**<sup>40</sup> Use a sample to contain about 0.05 gram of dry solids. Ash as for plasma by the method which follows, with addition of small amounts of ammonium nitrate if necessary. Complete

<sup>35</sup> L. Isaacs, *Bull. soc. chim. biol.* 6, 157-68 (1924).

<sup>36</sup> G. Bertrand, *Ibid.* 6, 656 (1924).

<sup>37</sup> J. H. Foulger, *J. Am. Chem. Soc.* 49, 431 (1927).

<sup>38</sup> Emil Truog and A. H. Meyer, *Ind. Eng. Chem., Anal. Ed.* 1, 136-9 (1929).

<sup>39</sup> F. Oberhauser and J. Schormüller, *Z. anorg. Chem.* 178, 381-8 (1929).

<sup>40</sup> Walter Parri and Giuseppi Scotti, *J. pharm. chim.* 18, 513-27 (1933).

as for serum. Give particular attention to elimination of interference by phosphorus in the procedure. This method was designed for hydroxylamine and hyposulfite reduction.

*Plasma.*<sup>40</sup> Measure 0.2 cc. into a platinum crucible. Add 0.2 gram of sodium bicarbonate. Heat gently at first, then strongly, until the fusion is complete. Moisten the residue carefully with 2 drops of 1:3 nitric acid. Add 1 cc. of 1:3 sulfuric acid to complete the solution.

**Procedure—Development with Sulfite.** Heat the solution to boiling. Neutralize with 10 per cent acetic acid and add 3 cc. in excess. Add 10 cc. of water, and 5 cc. of a 10 per cent ammonium molybdate solution. Transfer to a Pyrex tube calibrated at 25 cc.

At the same time put 1 cc. of a standard solution of silica, corresponding to 1 mg. of silica,<sup>44</sup> into a similar Pyrex tube, add 12 cc. of water, 3 cc. of 10 per cent acetic acid and 5 cc. of a 10 per cent ammonium molybdate solution. Put the two tubes in a boiling water bath for 5 minutes, remove and add 2 cc. of a saturated solution of sodium sulfite. Dilute each to 25 cc. Filter if necessary. After 15 minutes compare by dilution or by balancing. The color increases with time, but is still comparable after 24 hours.

By this method, if the sample contains blood, iron will interfere. Modify the standard to correspond. Put into a tube about 15 cc. of water, 3 cc. of 10 per cent acetic acid, 5 cc. of 10 per cent ammonium molybdate solution, and 1 cc. of a 1 per cent solution of ferric ammonium alum. Put in a boiling water bath for 5 minutes and add 2 cc. of a saturated solution of sodium sulfite. A yellow color develops. Add sufficient of this solution to the standard to match the yellow tint of the sample and compare sample and standard by dilution. As an alternative, dilute the standard to a definite volume and compare by balancing.

*Development with Hydroxylamine and Hyposulfite.*<sup>40,46</sup> Add 1 cc. of 10 per cent ammonium molybdate solution to the concentrated acid solution of silicate. Add 2 cc. of 95 per cent alcohol to prevent precipitation of the phosphomolybdate. Transfer this to a suitable tube using four 1 cc. portions of water. The solution should now be less than 10 cc. of a yellow liquid.

To prevent precipitation of phosphorus, add 1 cc. of a diammonium citrate solution made from 20 grams of citric acid crystals, 10 cc. of concentrated ammonium hydroxide and 10 cc. of water, or add 0.5 gram

<sup>44</sup> See p. 519.

<sup>46</sup> See p. 411, footnote 15.



of solid sodium citrate. This at once reduces the color of the phosphomolybdate, but not that of the silicomolybdate. The pH should be 4.0-4.2. At pH 6.8 the silicomolybdate color would also disappear. To obtain the final color, add at once to the cold solution 0.3 gram of hydroxylamine hydrochloride and 0.4 gram of sodium hyposulfite. Mix. The color develops at once. Compare with a standard. The resulting color is claimed to be a purer blue than the one produced with formaldehyde or with sodium sulfite. The standards will serve for 2 days if more sodium hyposulfite is added to revive the color.

*Development with Stannous Chloride.*<sup>39</sup> Add 10 cc. of a 10 per cent neutral solution of ammonium molybdate to the sample. Acidify by slowly adding 1:1 acetic acid. Prepare a 5 per cent solution of stannous chloride by dissolving 5 grams in about 50 cc. of water, adding 10 per cent sodium hydroxide solution until the precipitate first formed has completely dissolved, and diluting to 100 cc. Add this to the sample until a maximum color is developed. A few drops is usually sufficient but 20 cc. will not ordinarily cause error provided an equal amount is added to the standard used for comparison. The method is sensitive to 1 p.p.m.

#### SILICIC ACID BY PYRROL

Pyrrol gives a deep blue coloration with selenious and silicic acids.<sup>48</sup> In phosphoric acid this is sensitive to 2 p.p.m. The sensitivity is greatly increased by the presence of iron. Other ions probably interfere. The color fades quickly.

**Procedure**—To a sample of 2 cc. add 1 cc. of 5 per cent ferric chloride solution and dilute nearly to 10 cc. with syrupy phosphoric acid. Mix, add 10 drops of a 1 per cent solution of pyrrol in 95 per cent alcohol and complete the dilution to 10 cc. Compare with standards similarly treated.

As an alternative dilute the sample and ferric chloride to 7 cc. with 1:1 sulfuric acid, add 2 cc. of 8 per cent ammonium molybdate solution and complete the dilution with 1:1 sulfuric acid.

<sup>48</sup> R. Berg and M. Teitelbaum, *Mikrochem., Emich Festschr.* 1930, 23-6.

## CHAPTER LI

### BORON

#### BORIC ACID BY CURCUMIN

THE red color produced by boric acid when treated with oxalic acid and curcumin is especially adapted to estimation of the quantity of boric acid present in food.<sup>1</sup> The advantage is not in the speed but in the greater accuracy of the method over the gravimetric method. The weight of boric anhydride present may be as much as 2 mg.

**Sample**<sup>2</sup>—Place a 10–15 cc. sample in a platinum dish, make strongly alkaline with saturated barium hydroxide solution and evaporate to dryness. Char the residue, disintegrate it, acidify with 1:1 hydrochloric acid and extract with several successive portions of hot water. Filter the extracts, transfer the filter paper and contents to a platinum dish, make strongly alkaline with saturated barium hydroxide solution and ash. Carbon should practically disappear. Dissolve this ash in 1:1 hydrochloric acid, add to the original filtrate and dilute to 100 cc.

To 10 cc. of this solution add 10–15 grams of purified and ignited sand and make alkaline with saturated barium hydroxide solution. Evaporate to dryness at 105° with occasional stirring. Add enough 1:1 hydrochloric acid to make the contents of the dish faintly acid, then 2 cc. of a saturated solution of oxalic acid.

**Procedure**—Add 2 cc. of a 0.1 per cent alcoholic solution of curcumin. Mix the contents of the dish well and cover with a glass funnel whose stem is connected to a set of potash bulbs containing saturated barium hydroxide solution. Connect the other end of the set of potash bulbs to an aspirator. Place the dish on a paraffin bath at 105°, set the potash bulbs in water and gently aspirate until the mass is dry. Add 1 cc. of the curcumin solution to the dish and dry again.

Extract the contents of the dish with several portions of alcohol, decant the clear liquid and filter into a flask. Add the contents of the

---

<sup>1</sup> F. A. Gooch, *Am. Chem. J.* 9, 23-33 (1887).

<sup>2</sup> Charles E. Cassal and Henry Gerrans, *Chem. News* 87, 27 (1903).

bulbs to the residue in the dish. Dry, make acid with 1:1 hydrochloric acid, treat with reagents as for the original substance, and dry. Add the alcoholic extract from this to the previous extract. Two such extractions are usually sufficient.

**Standard**—Make a standard solution of pure boric acid by dissolving 0.1772 gram in water and diluting to 1 liter. This contains 0.1 mg. of boric acid anhydride,  $B_2O_3$ , per cc. Treat 10 cc. of this solution in the same manner as the sample. Compare by dilution of the sample with alcohol. The series of standards and duplication methods are unsatisfactory. If one tube apparently has more of an orange tint than the other it is due to the presence of an excess of curcumin and a drop or two should be added to the other tube until the colors are similar, after which dilution may be more accurately carried out. The rather elaborate precautions outlined are necessary to prevent loss of boric acid by volatilization.

#### BORIC ACID BY TURMERIC PAPER

The amount of boric acid in a sample may be estimated by treatment with methyl alcohol, distillation of the resultant methyl borate into alkali and, after conversion to boric acid, comparing the stain made with the curcumin in a standard turmeric paper and those made by known quantities of boric acid.

This modification of the usual gravimetric procedure<sup>3,4</sup> was originally developed for determination of boron in animal or vegetable tissues, using 10 or 1 gram respectively.<sup>5</sup> The method has been applied to very small amounts of boron in soils.<sup>6</sup> For general use the sample should be so chosen as to contain not over 0.1 mg. of boric acid anhydride.

**Sample**—Make the sample alkaline with sodium hydroxide solution and ignite in platinum until the organic matter is thoroughly charred. It is not necessary to burn out the carbon completely. Triturate the ash with 5 to 10 cc. of 85 per cent phosphoric acid and rinse into a 250 cc. flask fitted with a dropping funnel. Wash out the dish with 20 cc. of

<sup>3</sup> Th. Rosenblatt, *Z. anal. Chem.* 26, 18 (1887).

<sup>4</sup> F. A. Gooch, *Chem. News* 55, 7 (1887).

<sup>5</sup> G. Bertrand and H. Agulhon, *Bull. soc. chim.* [4] 7, 90 (1910); *Compt. rend.* 156, 732, 2027 (1913).

<sup>6</sup> Wilfred W. Scott and Sondheim K. Webb, *Ind. Eng. Chem., Anal. Ed.* 4, 180-1 (1932).

methyl alcohol and add to the solution in the flask. Distil from a steam bath, receiving the distillate in a platinum dish containing a few drops of *N* sodium hydroxide solution. Add 10 cc. more of methyl alcohol to the flask through the dropping funnel and distil this into the original distillate. This will usually carry with it the last traces of methyl borate. Add 10 cc. more of methyl alcohol through the dropping funnel and distil. Add 10 cc. more of alcohol and start distillation again. Test a drop with turmeric paper and hydrochloric acid to make sure that the boron is already completely distilled, or if any is coming over, complete the distillation and add to the solution.

*Soil.*<sup>6</sup> Dry the sample in an electric oven at 105° for 24 hours. Pulverize and sift out foreign matter. Mix 100 grams with 100 cc. of boiling water and let settle for 30 minutes. For clay or other soils which settle slowly either extend this to several hours or add 1 gram of salt. Filter into a silica dish. From this point on continue as for the sample above.

**Test Paper**—Cut photographic or drafting paper of uniform texture to 45 by 3 mm. strips. Soak for 5 to 10 minutes in a freshly filtered 1 to 1.5 per cent solution of curcumin in alcohol and dry. An extract of powdered turmeric may also be used. The paper will be more or less brown but must be uniform in color. It is essential that the papers be of uniform thickness, width and saturation with the reagent.

**Procedure**—Evaporate the distillate to dryness, cool, and add 4 drops of 1:1.5 hydrochloric acid and 0.5 cc. of water. Wash this into a 10 × 30 mm. tube and dilute to 1.5 cc. Transfer the standards to similar vials. Immerse a strip of the test paper in each vial to a depth of 15 mm. and expose the vials to a temperature of 35° for 3 hours. If time is not a consideration the vials may be allowed to stand at room temperature for 24 hours after which the results will be similar to those obtained by heating. The length of the red color produced on the paper is a measure of the amount of boric acid present in the sample. The color of the standard papers so prepared is permanent and by protecting them from dust they may be kept several months.

Suitable amounts of boron with the lengths of coloration are as noted in the following table.

Milligrams of boron	Grams boric acid anhydride	Length of coloration
0.1	0.00032	8 mm.
0.05	0.000164	7 mm.
0.01	0.000032	5 mm.
0.005	0.000016	3 mm.
0.001	0.0000032	2 mm.
0.0005	0.0000016	1.5 mm.
0.0000	0.0000000	Brown only

The strip showing the coloration of the sample should be exposed to ammonia fumes after it has been matched. A blue color confirms the coloration as being due to boron. A blank test should show no blue with ammonia. If the alcohol gives a positive test for boron it should be made alkaline and distilled.

**Standards**—Prepare a boric acid standard containing 0.5638 gram per liter. This contains 0.01 mg. of boron per cc. Prepare weaker standards by dilution of 10 cc. of this to 100 cc. and to 1 liter. To varying amounts of these standards add four drops of 1:1.5 hydrochloric acid and make each to 1.5 cc.

#### BORON BY COMPARISON OF THE STAINS PRODUCED WITH TURMERIC

This method <sup>8</sup> is a simple modification of the previous procedure for less accurate estimation.

**Procedure**—Add 1 cc. of 1:24 hydrochloric acid and 1 cc. of 1 per cent turmeric solution to a 10 cc. sample in a porcelain evaporating dish. Evaporate to dryness slowly on a water bath. If boron is absent the residue will not be pink. If boron is present the pink or rose color may be used for estimation of the amount by comparison with a series of standards.

<sup>8</sup> Margaret D. Foster, *Ind. Eng. Chem., Anal. Ed.* 1, 27-8 (1929).

**Standards**—Prepare solutions of boric acid containing 0.01 and 0.1 gram per liter. Prepare standards in which 0.25, 0.50, 0.75 and 1 cc. of the more dilute solution are used. Also prepare a series using 0.1, 0.3, 0.6, 1.0, 2.5 and 5.0 cc. of the more concentrated solution. These standards are treated in the same way as the sample.

Frequently the standards are not comparable with the sample because of differences in salt content. In that case add a solution containing 2.5 per cent each of sodium chloride and sodium sulfate and try to get a similar deposit in each.

## CHAPTER LII

### CHLORIDES

#### CHLORIDES NEPHELOMETRICALLY AS SILVER CHLORIDE

THE classical method for the determination of traces of chlorides is nephelometrically as silver chloride.<sup>1,2,3,4</sup> The method is widely applicable for accuracy varying from rough estimation to accurate determination. A suitable concentration is 0.2 mg. per 100 cc. of sample after dilution. Other ions forming insoluble silver salts, such as bromides and iodides must be absent.

Possible sources of error in the equal-opalescence end-point method as used in atomic weight work have been discussed by Johnson.<sup>5</sup> In this work the supernatant liquid over silver chloride is compared in two tubes, one containing excess silver, the other an equivalent excess of chloride. One sol is positive by adsorption of silver ions, the other negative by adsorption of halide ions. When multivalent ions are present in addition their coagulating effect on these 2 sols may be quite different.

The subject of errors of the method has been discussed in greater detail under silver. Results are reproducible to 2 to 3 per cent.

**Sample—Solutions.** Select a sample which contains approximately 0.2 mg. of chloride. As standard for balancing take 10 cc. of solution containing 0.02 mg. of chloride per cc. and dilute to 20 cc.

**Phosphoric acid.**<sup>6</sup> Weigh 100 cc. of the acid and dilute with water to 1 liter. Take an aliquot corresponding to 2 grams of actual phosphoric acid and dilute to 20 cc. As standard for balancing take 10 cc. of solution containing 0.02 mg. of chloride per cc., add 2 cc. of C.P. phosphoric acid and dilute to 20 cc.

<sup>1</sup> T. W. Richards, *Proc. Am. Acad. Arts Sci.* 30, 385 (1894).

<sup>2</sup> T. W. Richards and R. C. Wells, *Am. Chem. J.* 31, 235-43 (1904); *J. Am. Chem. Soc.* 27, 483 (1905).

<sup>3</sup> R. C. Wells, *Am. Chem. J.* 35, 99-114 (1906).

<sup>4</sup> T. W. Richards, *Ibid.* 35, 510-13 (1906).

<sup>5</sup> Clyde R. Johnson, *J. Phys. Chem.* 35, 540-2, 830-5, 2237-44, 2581-4 (1930).

<sup>6</sup> William H. Ross, C. B. Durgin and R. M. Jones, *J. Ind. Eng. Chem.* 14, 533-5 (1922).

Repeat, varying the proportions based on this first estimation, for more accurate results.

**Procedure**<sup>7</sup>—*Balancing.* Add 10 cc. of 0.1 *N* nitric acid, mix and add 10 cc. of a 0.005 *M* silver nitrate solution. Mix and place in a water bath at 40° for 30 minutes or more. Cool rapidly to room temperature and compare within 30 minutes in a nephelometer with a suitable standard similarly prepared. It is advisable to rinse out the nephelometer tubes first with distilled water and then with the solutions to be introduced. The eyepiece of the instrument should be kept in exactly the same position throughout a series of comparisons.

If maintained at 20° the opalescence increases slowly to an almost constant value in an hour. Heating to 60° not only gives full development in less time but results in a greater opalescence, which falls off on continued heating. After 30 minutes coagulation begins. Heated at 40° this coagulation does not occur and constant opalescence is obtained after 30 minutes. Further heating at this temperature has no effect. The solution must be cooled before reading. Exposure to diffused daylight has a perceptible effect, especially on the more concentrated solutions. If greater sensitiveness is desired dilute to 100 cc. with chloride-free alcohol, the final solution containing about 50 per cent of alcohol.

*Duplication.*<sup>7a</sup> Transfer the sample to one cylinder and an equal volume of water containing the same known impurities to another cylinder. Dilute each to 20–30 cc. and add 5 cc. of concentrated nitric acid and 2 cc. of 0.1 per cent gelatin solution. Dilute the sample to 49 cc. and add 1 cc. of 0.1 *N* silver nitrate solution and mix. Dilute the standard to about 45 cc. and add 1 cc. of 0.1 *N* silver nitrate solution. Add the standard sodium chloride solution to this standard until the opalescence of the standard matches that of the sample, comparing them against a black background. Adjust the volume as usual.

**Standard**—Dissolve 0.3297 gram of sodium chloride in water and dilute to 100 cc. This contains 2.0 mg. of chloride per cc. Dilute 10 cc. of this solution to 1 liter. It then contains 0.02 mg. per cc. A 10 cc. portion is suitable for use as a standard.

<sup>7</sup> A. B. Lamb, P. W. Carleton and W. B. Meldrum, *J. Am. Chem. Soc.* 42, 253 (1920).

<sup>7a</sup> G. T. Mikhalechishin, *Zavodskaya Lab.* 1933, No. 8, 14-16.



## CHLORIDES BY DETERMINATION OF SILVER IN SILVER CHLORIDE

As an alternative to the preceding method an excess of silver nitrate may be added, the resulting silver chloride separated, dissolved and the silver in it estimated colorimetrically by treatment with hydrogen sulfide or sodium sulfide. The silver may also be estimated as the ricinoleate.<sup>8</sup>

**Sample** <sup>10</sup>—*Urine*. Transfer 1 cc. of urine to a centrifuge tube. Add about 5 cc. of water, 1 cc. of 1:3 nitric acid and 2 cc. of 10 per cent silver nitrate solution. Mix well and centrifuge. Decant and make sure that the supernatant liquid shows no test for chloride. Wash the precipitate 3 times with 5 cc. of distilled water.

**Procedure**—Add 1 cc. of 1:10 ammonium hydroxide to the residue, stir, and centrifuge again. Transfer the supernatant liquid containing the silver chloride to a 100 cc. volumetric flask, wash the residue and dilute to about 80 cc.

In a similar flask put 10 cc. of a solution of silver nitrate containing 1.4535 grams per liter. Each cc. is equivalent to 0.5 mg. of sodium chloride or 0.304 mg. of chloride. Add to this standard 2 cc. of 1:10 ammonium hydroxide and 50 cc. of water. Add to each 1 cc. of a 10 per cent gelatin solution and 2 cc. of a 10 per cent sodium sulfide solution or saturated hydrogen sulfide solution. After a minute dilute each to 100 cc. and compare in a colorimeter.

The method is also applicable to micro determination of amounts under 0.1 mg.

## CHLORIDES NEPHELOMETRICALLY BY DETERMINATION OF EXCESS SILVER

This method <sup>11</sup> is similar to that preceeding. The amount of silver nitrate is measured and the excess silver determined after separation of silver chloride.

**Procedure**—To a 10 cc. sample, free from protein, add silver nitrate solution containing 0.4763 grams per liter. Each cc. is equivalent to 0.1 mg. of chloride. Add the amount required to react and about 5 cc. in excess. Centrifuge to remove silver chloride and decant the clear layer. Wash twice with 5 cc. of distilled water and add the washings to the

<sup>8</sup> T. v. Heidelberg, *Biochem. Z.* 192, 238-40 (1928).

<sup>10</sup> S. Yoshimatsu and H. Sakurada, *Tôhoku J. Exptl. Med.* 8, 107-12 (1926).

<sup>11</sup> Ricardo Calatroni and Emilo Tschopp, *Biochem. Z.* 208, 274-8 (1929).

solution first decanted. Transfer to a 50 cc. volumetric flask. Add 1 cc. of 10 per cent gelatin solution, mix well and add 2 cc. of a saturated solution of hydrogen sulfide. Dilute to volume.

Estimate colorimetrically in comparison with 5 cc. of the standard silver nitrate solution, each cc. of which is equivalent to 0.1 mg. of chloride, similarly treated. By difference estimate the amount of silver precipitated as silver chloride and therefore the amount of chloride present in the sample.

#### TURBIDIMETRIC ESTIMATION OF CHLORIDE

By comparison with prepared stable turbidity standards a rough estimation of the amount of chloride present can be obtained.<sup>12</sup>

**Procedure**—To a 10 cc. sample made slightly acid with nitric acid add 0.005 *N* silver nitrate solution until the opalescence no longer increases. Compare the turbidity with an artificial standard turbidity such as that of the German Pharmacopoeia or American Public Health Association. Estimate the chloride content from data obtained by comparing standard chloride solutions with the turbidity standards. A high degree of accuracy is not to be expected.

#### CHLORIDES INDIRECTLY BY SILVER CHROMATE

An indirect method for determining chlorides in blood depends on the action of silver chromate.<sup>13</sup> By reaction silver chloride is precipitated and leaves a yellow sodium chromate solution. This may be estimated either by duplication or balancing. Results by this method differed from those by the method of Whitehorn<sup>14</sup> by an average of less than 1 per cent.

**Sample**—*Blood*.<sup>15</sup> The method is applicable to human, beef, chicken, rabbit or dog blood and probably to others. Transfer 5 cc. of the sample of blood to a 100 cc. volumetric flask. Dilute with 35 cc. of water and mix. Add 5 cc. of a 10 per cent solution of commercial sodium tungstate and mix. Add 3.5 cc. of *N* sulfuric acid, close with a rubber stopper and

<sup>12</sup> S.

and T. Daigo, *J.*

*Soc. Japan* 48, 702-12 (1928).

*Biol. Che*

, *J. Biol.*

Wu, *J. J*

shake vigorously a few times. Only a few air bubbles should form as a result of shaking.

Not over 10 mg. of potassium oxalate should be present in the sample. Citrate must be absent. If coagulation has proceeded properly the precipitate changes slowly from pink to dark brown. If the dark brown color does not appear, too much oxalate is present or citrate was used as an anticoagulant in the sample. In that case add *N* sulfuric acid drop by drop, shaking vigorously after each addition, until coagulation is complete. Wet a filter large enough to contain the entire contents of the flask, with a few cc. of the solution. Pour the contents into the filter and cover to prevent evaporation. If the filtrate is not perfectly clear return the first few cc. to the funnel. The filtrate so obtained should be neutral or just faintly acid to Congo red paper. It can be preserved for several days by addition of 1-2 drops of toluene. Alcohol, heat and Dazol may also be used for deproteinizing the blood to be used by this method.<sup>16</sup>

**Silver Chromate Reagent**—The silver chromate should be the red form. If necessary to prepare it add 200 cc. of 5.5 per cent potassium chromate solution slowly to 100 cc. of boiling 10 per cent silver nitrate solution. The precipitate will settle rapidly. Continue to add chromate solution drop by drop until it is in slight excess as shown by a yellow color in the supernatant liquid. Filter on a Büchner funnel, wash with distilled water and air-dry. Unless the solution is boiling while silver chromate is precipitated, the bichromate will form.<sup>17</sup>

**Procedure**—Pipet 10 cc. of the blood filtrate into a centrifuge tube. Add about 0.1 gram of magnesium carbonate to neutralize a possible trace of excess acidity. Stir and add about 0.05 gram of dry silver chromate. If all the red particles disappear add more. Centrifuge for 2 minutes. Decant carefully through a filter paper into a 25 cc. volumetric flask. Add 10 cc. of water to the residue in the tube and centrifuge for 5 minutes. Filter into the flask. If the filtrate is slightly turbid with silver chloride add 1 cc. of 1:20 ammonium hydroxide. Dilute to 25 cc.

The centrifuging operation may be eliminated and time saved by direct filtration of the suspension of silver chromate after the color has

<sup>16</sup> Shin-ichi Yoshimatsu, *Tôhoku J. Exptl. Med.* 7, 553-9 (1926).

<sup>17</sup> M. Duprey, *J. Biol. Chem.* 58, 675-9 (1923-4).

been developed.<sup>18</sup> A good quality paper should be used, and to avoid too much wash-water being required, a 4 cm. size is recommended.

*Estimation by Duplication.* Transfer the sample to a comparison tube. Into a second 25 cc. comparison tube put 5 cc. of 0.02 *N* sodium chloride solution and dilute nearly to volume. Add a standard solution containing 0.2738 gram of potassium chromate per liter, until the color matches that of the sample. Each cc. of this solution is equivalent to 0.1 mg. of chloride. Adjust the volume of standard to match that of the sample.

*Estimation by Balancing.* Prepare a 25 cc. standard containing 5 cc. of 0.02 *N* sodium chloride solution together with a known amount of the above potassium chromate solution so that the color nearly matches that of the sample. Dilute exactly to volume and compare by balancing. As yellow colors are rather hard to match, a blue glass may be interposed.<sup>19</sup>

#### CHLORIDES THROUGH SILVER CHROMATE BY DEVELOPMENT AS THE IODIDE

The previous method has been modified to determine the chromate present by the amount of iodine which it liberates in acid solution.<sup>17</sup> A modification recently proposed<sup>21</sup> determines the liberated chromate by diphenylsemicarbazide. As a micro method, it is stated to be accurate to 3 per cent on 0.0004 mg. of sodium chloride.

**Sample**—Same as previous method.

**Procedure**—Follow the previous method until the solution of chromate equivalent to the chloride is obtained. Centrifuge, decant and filter, collecting the filtrate in a 25 cc. volumetric flask. The volume of filtrate and washings must not exceed 22 cc. Add 1 cc. of 50 per cent potassium iodide solution and 1 cc. of 1:10 sulfuric acid. Dilute to 25 cc. with distilled water and mix well.

<sup>18</sup> John H. Yoe, "Photometric Chemical Analysis," 1st ed., vol. I, "Colorimetry," p. 162. John Wiley and Sons, New York, N. Y. (1928).

<sup>19</sup> L. Michaelis, *Deut. med. Wochschr.* 47, 465 (1921).

<sup>21</sup> B. B. Westfall, *Am. J. Med. Sci.* 185, 148 (1933).

Take a known volume of standard chloride solution and carry it through the same procedure at the same time. Develop the color in the same way. A standard chromate may also be used but the results are not as accurate, since errors of procedure and individual errors, which otherwise tend to counterbalance, may occur. The maximum color is attained in a few seconds and remains unchanged for many hours.

**Standard Chromate Solution**—If this is to be used dissolve 0.8350 gram of potassium chromate in water and dilute to 1 liter. The value is approximately that of a sodium chloride solution containing 0.5 mg. per cc. Before use an exact value must be obtained by comparison against a standard sodium chloride solution.

## CHAPTER LIII

### CHLORINE AND CHLORAMINE

#### CHLORINE BY *o*-TOLIDINE

IN THE estimation of free chlorine, as contrasted with methods for chlorides, the most common reagent is *o*-tolidine. In acid solution this produces a yellow color which may be estimated when only 0.01 p.p.m. of chlorine is present.<sup>1</sup> The usual application is to chlorination of water.<sup>2</sup> The color is stable for only a comparatively short time. At the end of an hour it has faded about 50 per cent. The action is an oxidation-reduction one, so that oxidizing agents, reducible substances, and unstable chlorine addition-products behave similarly. Ferric salts produce some color on standing, but for practical purposes they do not have to be considered.<sup>3</sup> Manganese present as manganic hydroxide gives the same reaction as chlorine,<sup>4</sup> 0.05 and 2.0 p.p.m. of the former giving a color equivalent to 0.03 and 0.5 p.p.m. of free chlorine.<sup>5</sup>

A standard solution of chlorine is not satisfactory as the results are inconsistent among themselves<sup>5</sup> unless great care is used. Several manufacturers offer kits with permanent sealed standards for field and factory use.

***o*-Tolidine Reagent**<sup>7</sup>—Transfer 1 gram of *o*-tolidine, m.p. 129° to a mortar. If necessary prepare this by extracting the commercial product with water in a Soxhlet apparatus. Add 5 cc. of 1:5 hydrochloric acid. Grind to a thin paste and add 150 to 200 cc. of distilled water. The *o*-tolidine goes into solution at once. Transfer to a 1000 cc. graduate and dilute to 505 cc. with distilled water. Make up to 1000 cc. with 495 cc.

---

<sup>1</sup> J. W. Ellms and S. J. Hauser, *J. Ind. Eng. Chem.* 5, 915 (1913).

<sup>2</sup> American Public Health Association, "Standard Methods for the Examination of Water and Sewage," pp. 44-5 (1925).

<sup>3</sup> O. Forsberg, *J. Am. Water Works Assoc.* 15, 706 (1926).

<sup>4</sup> E. S. Hopkins, *Ind. Eng. Chem.* 19, 744 (1927).

<sup>5</sup> H. F. Muer and F. E. Hale, *J. Am. Water Works Assoc.* 13, 50-69 (1925).

<sup>7</sup> E. J. Theriault, *J. Am. Water Works Assoc.* 18, 125 (1927); *Public Health Reports*, 42, 668-72 (1927).

of 1:5 hydrochloric acid. The hydrochloric acid must not exceed 178 cc. of the concentrated solution per liter.<sup>8</sup>

**Procedure**—*Water, less than 1.0 p.p.m.* Add 1 cc. of the *o*-tolidine reagent to 100 cc. of the sample. Mix well in a Nessler tube and compare after 5 minutes with a series of permanent artificial standards.

*Water, more than 1.0 p.p.m.* Add 5 cc. of the *o*-tolidine reagent to 100 cc. of the sample. Mix well in a Nessler tube and compare after 15 minutes with a series of permanent artificial standards.

*Sewage.*<sup>9</sup> To a sample of 20 cc. add 5 cc. of concentrated hydrochloric acid and 1 cc. of reagent. Compare with suitably calibrated standards. At least 30 minutes contact with chlorine must be allowed before analyzing.<sup>10</sup>

*Water or Sewage. Interfering substances present.* A method of correction<sup>11</sup> is to boil 100 cc. of the chlorinated sample down to about 75 cc. When cool, dilute to volume. Determine the chlorine value of the interfering substance and subtract from the value obtained on the same sample which was not boiled.

Another method, if interfering substances are present, is to sample before and after chlorination. Shake the unchlorinated sample with oxygen. Determine the chlorine value of the interfering substance and subtract from the total value obtained after chlorination.

*Air.*<sup>12</sup> Place 10 cc. of *o*-tolidine reagent in a test tube. Close the tube with a 2-holed rubber stopper fitted with a long entrance tube ending in a capillary extending beneath the surface of the liquid, and a short exit tube. Connect the short exit tube to a tube entering a large bottle. Fit this with a 2-holed rubber stopper, containing as a second tube, a long exit tube extending nearly to the bottom of the bottle. Fill the bottle with water to serve as a siphon and measure the volume of water siphoned off to estimate the volume of air drawn through the solution of reagent. Aspirate from 50 to 500 cc. of air through the solution of reagent at a moderate rate of speed. Rinse into a Nessler tube and dilute to 100 cc. Compare with standards at the end of 5 minutes.

<sup>8</sup> C. S. Bornuff, S. J. Vellenge and R. H. Phelps, *J. Am. Water Works Assoc.* 20, 404-6 (1928).

<sup>9</sup> Linn H. Enslow, *Md. Water and Sewage Assoc.*, April, 1928, 39-49.

<sup>10</sup> C. M. Nichols, *Proc. 13th Ann. Meeting of N. J. Sewage Works Assoc.*, Papers on Sewage Chlorination 1-2.

<sup>11</sup> Linn H. Enslow, *Water Works and Sewerage* 78, 183-4 (1931).

<sup>12</sup> L. E. Porter, *Ind. Eng. Chem.* 18, 730 (1926).

TABLE 5. STANDARDS FOR CHLORINE WITH *o*-TOLIDINE

Volume of Standards to be Diluted to 100 cc.

Chlorine p.p.m.	Bichromate Solution cc.	Copper Sulfate Solution cc.
0.01	0.18	0.3
0.02	0.32	0.5
0.04	0.61	1.0
0.06	0.87	1.4
0.08	1.1	1.7
0.10	1.3	1.9
0.15	1.7	1.9
0.20	2.1	2.0
0.25	2.6	2.0
0.30	3.0	2.0
0.35	3.4	2.0
0.40	3.8	2.0
0.50	4.7	2.0
0.60	5.5	2.0
0.70	6.4	2.0
0.80	7.2	2.0
0.90	8.1	2.0
1.00	9.0	2.0

Compare with color developed by 1 cc. of *o*-tolidine reagent in 5 minutes.

1	9	
2	16	
3	22	
4	28	
5	33	
6	38	
7	44	
8	50	8
9	57	8
10	66	8

Compare with color developed by 5 cc. of *o*-tolidine reagent in 15 minutes.



**Standards**<sup>5</sup>—*Artificial*. Prepare a solution of 1.5 gram of crystallized copper sulfate and 1.0 cc. of concentrated sulfuric acid in distilled water and dilute to 100 cc. Dissolve 0.25 gram of potassium bichromate and 1.0 cc. of concentrated sulfuric acid in distilled water and dilute to 100 cc. For accuracy in measurement for standards up to 0.1 p.p.m. of chlorine each of these should be diluted to 0.1 strength with distilled water, and 10 times the quantity listed should then be used. Prepare the series of artificial standards with these solutions according to the accompanying Table 5.

*Chlorine*. Mix the specified amount of a chlorine solution of known strength with the necessary volume of distilled water. Add the volume of *o*-tolidine reagent in a Nessler tube exactly as in preparation of the sample.

#### CHLORINE BY BENZIDINE

Free chlorine in the absence of permanganate ion can be estimated from the bright green color obtained by reaction with benzidine hydrochloride.<sup>14</sup> The color is much more intense and favorable for colorimetric comparison than that by the reaction of chlorine with *o*-tolidine. The color fades in about 2 minutes to an unstable greenish yellow. Comparison is therefore either with a fresh series of standards or more conveniently with artificial standards. Large amounts of sulfate interfere because of formation of insoluble benzidine sulfate.

**Procedure**—Measure a portion of sample to contain between 0.001 and 0.01 mg. of free chlorine. Dilute to 100 cc. and add one drop of benzidine reagent. This is a solution of 2.3 grams of benzidine per 100 cc. of 5 per cent hydrochloric acid. Mix and compare at once with the artificial standard by balancing.

**Standard**—The standard is prepared empirically to match a solution containing 0.003 mg. of free chlorine per 100 cc. Mix a 15 per cent solution of copper sulfate and a 0.5 per cent solution of picric acid until the desired tint of blue-green is obtained. Dilute this until it matches

<sup>14</sup> R. C. Stratton, J. B. Ficklen and W. A. Hough, *Ind. Eng. Chem., Anal. Ed.* 4, 2 (1932).

a freshly prepared 0.003 mg. free chlorine sample treated according to the recommended procedure.

#### CHLORINE BY DIMETHYL-*p*-PHENYLENEDIAMINE

Free iodine, bromine or chlorine gives a red color with dimethyl-*p*-phenylenediamine.<sup>15</sup> Since iodine and chlorine solutions of the same normality have the same color intensity, a chlorine solution of unknown strength may be compared with a standard iodine solution.

**Procedure**—To 50 cc. of solution to be examined add 1 cc. of 50 per cent acetic acid and 2 cc. of 2 *N* sodium acetate solution. Then add 10 drops of a 0.1 per cent alcoholic solution of dimethyl-*p*-phenylenediamine and dilute to 100 cc. The color develops in 10 to 15 minutes and must not stand over 30 minutes before comparison.

**Standards**—*Iodine*. Prepare a standard iodine solution containing 0.1269 gram per liter. This is equivalent to 0.03546 mg. of chlorine per cc. Treat 10 cc. of this diluted to 50 cc. at the same time the sample is treated and compare by dilution.

*Methyl red*.<sup>16</sup> Prepare a solution of 0.115 gram of methyl red per liter in 0.01 *N* hydrochloric acid. Each cc. is equivalent to 0.02 mg. of chlorine for a 50 cc. sample. Compare with the sample by duplication after 15 minutes has been allowed for development of the color.

#### CHLORINE BY STARCH-IODIDE SOLUTION

The method<sup>17</sup> is of historical interest but is inferior to the other methods quoted. It is therefore not given.

#### CHLORAMINE BY NESSLER'S REAGENT

Chloramine exists in solution at a pH above 4.4. Above 8.5 only the monochloramine is present, between 4.4 and 8.5 both mono- and dichloramine are present.<sup>18</sup> The chloramine content may be measured by acidifying one sample and rendering another alkaline. In the acid solution chloramine is converted to give two-thirds of the nitrogen as ammonia

<sup>15</sup> I. M. Kolthoff, *Chem. Weekblad* 23, 203-4 (1926).

<sup>16</sup> Knut Alfthan, *Finska Kemistsamfundets Medd.* 36, 109-12 (1928).

<sup>17</sup> Linn H. Enslow, *Proc. 2nd Ann. Conf. Maryland Water and Sewerage Assoc.* 39-49 (1928).

<sup>18</sup> R. M. Chapin, *J. Am. Chem. Soc.* 51, 2112 (1929).

and the balance as nitrogen trichloride. The ammonia formed may then be estimated by Nessler's reagent.<sup>19</sup> The ammonia in the alkaline solution serves as a correction for the ammonia originally present in the solution. The color is 90 per cent developed within 1 minute, after which destruction in the alkaline tube alters the results. The method is not as sensitive as the *o*-tolidine method. Probably even greater accuracy would be obtainable by reading the alkaline tube within 1 minute and the acid tube in 5 to 10 minutes.

**Procedure**—Transfer 50 cc. of sample to each of two 50 cc. Nessler tubes. Add 0.2 cc. of 1:10 sulfuric acid to one, to lower the pH below 4.4. Mix and let stand for 1 minute. If the pH of the original solution is below 8.6 add 0.1 cc. of 5 per cent sodium carbonate solution to raise the pH. To each add 2 cc. of Nessler's reagent.<sup>20</sup> Invert and compare with standards<sup>21</sup> within 1 minute. The difference in ammonia by the 2 readings gives chlorine as chloramine by the following formula:

$$\text{Ammonia} \times 3/2 \times 35.4/14 = \text{chlorine as chloramine.}$$

The value of the factor is 3.8.

#### CHLORAMINE BY *o*-TOLIDINE

The usual *o*-tolidine method for free chlorine<sup>22</sup> can be used to estimate chloramine. Nitrite ions interfere and the results are usually questionable.<sup>23</sup>

<sup>19</sup> Paul C. McNamee, *Ind. Eng. Chem., Anal. Ed.* 7, 233-4 (1935).

<sup>20</sup> Jackson's modification, see pp. 653-4.

<sup>21</sup> See p. 656.

<sup>22</sup> See pp. 538-41.

<sup>23</sup> R. Hulbert, *J. Am. Water Works Assoc.* 26, 1638 (1934).

## CHAPTER LIV

### BROMIDES

#### BROMIDES BY CHLORINE-WATER

BROMIDES may be determined by means of the red color of free bromine liberated by a very small amount of chlorine-water.<sup>1</sup> Iodide should not be present in a concentration over 50 per cent of that of the bromide and preferably not over 30 per cent. When iodide is low, accuracy to 1 per cent is attainable.

**Procedure**—Take a suitable volume of sample solution in a 100 cc. Nessler tube. This will desirably contain about 5 mg. of bromide. Prepare a standard by taking a suitable volume of a solution of potassium bromide containing 0.7446 gram per liter. Each cc. is equivalent to 0.5 mg. of bromine. Ten cc. of this standard will normally be used.

Dilute sample and standard to about 75 cc. Add 10 cc. of 1:1 sulfuric acid and mix. Add a saturated solution of chlorine dropwise to each until a maximum color is obtained. This will normally require 5.0–5.1 cc. If the brown color of iodine appears, add more chlorine-water until the interfering color is removed. Dilute each to 100 cc.

Balancing methods are permissible if the standard and sample nearly match. The standard must be fresh, as it fades on standing. The accuracy can be improved by adding to the sample an equal weight of the same kind of material being tested, known to be free from bromides.

**Standard**—Dissolve 0.7446 gram of sodium bromide in water and dilute to 1 liter. Each cc. contains 0.5 mg. of bromine. For more dilute standards dilute 20 cc. to 100 cc. This solution contains 0.1 mg. of bromine per cc. Dilution of this to 1 liter gives 0.01 mg. of bromine per cc.

#### BROMIDES BY SCHIFF'S REAGENT

Schiff's reagent, the color of which has been developed with a hypochlorite solution, gives a color with bromine suitable for colorimetric

---

<sup>1</sup> W. J. Dibdin and L. H. Cooper, *Analyst* 35, 159-61 (1910).

estimation by the series of standards method.<sup>2</sup> Accuracy to 5 per cent is claimed.

**Sample—Inorganic.** Dissolve a suitable amount of sample so that 1 cc. contains about 0.2 mg. of bromide. Alkaline fusion methods are permissible, provided no oxidizing agent is added for getting the sample into solution.

**Organic.** Ash without addition of oxidizing agents. Dissolve the ash and dilute to a volume in which the bromide concentration will be about 0.2 mg. per cc.

**Reagents—**Prepare a 1 per cent fuchsin solution. Add 80–90 mg. of sulfur dioxide dissolved in water, to 100 cc. of this solution and dilute to 1 liter. It will become colorless or faintly yellow in 24 to 36 hours and will keep for some days if cool and in the dark. Prepare an oxidizing solution by dissolving 1 gram of calcium hypochlorite containing about 0.4 gram of available chlorine in water and dilute to 500 cc. Mix 0.1 cc. of 1.49 per cent potassium bromide solution and 1 cc. of 1:4 sulfuric acid. To this mixture in 3 separate tubes, add 0.2, 0.25 and 0.3 cc. of the oxidizing solution. Add 1 cc. of Schiff's reagent to each of these. The oxidizing solution should be of such a concentration that one of them will have a grey-violet color. That is the standard volume of oxidizing solution to add. If necessary dilute the oxidizing solution to fall within that range.

**Procedure—**Measure 0.1, 0.2, 0.5, 0.8 and 1.0 cc. of the unknown into separate test tubes. To each add 1 cc. of 1:4 sulfuric acid, the standard volume of oxidizing agent and 1 cc. of Schiff's reagent. One should be grey-violet and contains about 0.1 mg. of bromide in the volume taken. Check this by taking the same amount of the sample in three tubes. Treat in the same way except that one receives 0.05 cc. less of oxidizing agent and should be yellow. One should duplicate the previous grey-violet. A third should have 0.05 cc. more of oxidizing agent and should be distinctly violet.

Prepare a series of standards containing 0.075, 0.1 and 0.125 mg. of bromide. Dilute each to the volume of the sample solution. To each add the same reagents as to sample which gave a grey-violet color and compare with that sample.

---

<sup>2</sup> E. Oppenheimer, *Arch. exp. Path. Pharmacol.* 89, 17-28 (1921).

## BROMIDES BY FUCHSIN

Bromine, liberated from bromides by chlorine-water, forms a violet color in a sulfuric acid solution of basic fuchsin. The intensity of color is proportional to the amount of bromine present.<sup>2a,b</sup> For estimation the color is extracted with isoamyl alcohol. The method will detect 0.007 mg. of bromine. The sample and standard should not differ by more than 50 per cent.

**Sample—Blood.** Measure 10 cc. of blood into a 50 cc. porcelain dish. At the same time measure 4 cc. of standard sodium bromide solution containing 0.01 mg. of bromide per cc.,<sup>2c</sup> into a similar dish. To each add 5 drops of 10 per cent potassium hydroxide solution. Mix well and add 0.5 gram of magnesium oxide to each. Be sure that the magnesium oxide covers the entire surface. Heat to dryness cautiously over a burner and ash in a muffle. Dissolve the ash in about 30 cc. of 7.5 per cent sulfuric acid and dilute each to 40 cc.

**Reagent—**Place 100 cc. of 5 per cent sulfuric acid in a 250 cc. cylinder. Add 10 cc. of a 1 per cent fuchsin solution and shake. Let stand for 2 hours at which time the fuchsin should be entirely colorless.

**Procedure—**Transfer 20 cc. of the sample and standard solutions to separatory funnels. Add 2 cc. of fresh 5 per cent chlorine-water and 20 cc. of the fuchsin reagent. Mix vigorously and add 5 cc. of isoamyl alcohol. Shake and separate the isoamyl alcohol layers for comparison.

## BROMIDES BY GOLD CHLORIDE

A solution of gold chloride gives a variable color with solutions of bromides, the color varying with the concentration of bromide.<sup>3,4</sup> The method has been worked out for use with blood samples to guard against bromide intoxication. The error is less than 5 per cent.

**Procedure—**Draw 10 cc. of blood from a vein and allow it to clot.

---

<sup>2a</sup> Roberto Indovina, *Biochem. Z.* 275, 286-92 (1935); *Boll. soc. ital. biol. sper.* 10, 189-91 (1935).

<sup>2b</sup> R. Casares Lopez, *Farm. moderna* 46, 55-7 (1935); *Ann. fals.* 28, 115-6 (1935).

<sup>2c</sup> See p. 544.

<sup>3</sup> Otto Wuth, *J. Am. Med. Assoc.* 88, 2013-8 (1927).

<sup>4</sup> W. A. Taylor, *J. Lab. Clin. Med.* 13, 495-6 (1927-8).

To 2 cc. of the serum add 4 cc. of water and 1.2 cc. of 20 per cent trichloroacetic acid. Shake, let stand for 20 minutes and filter. If the filtrate is not clear, filter again. To 2 cc. of filtrate add 0.4 cc. of 0.5 per cent gold chloride solution. Shake and compare with a series of standards.

**Series of Standards**—Prepare a solution containing 1 gram of sodium bromide and 0.15 cc. of a 0.1 per cent solution of potassium iodide per liter. The iodide results in a more comparable color, since it corrects for the normal iodide content of the body fluids.<sup>5</sup> To 2 cc. portions of distilled water or, better, bromide-free serum, add 0.30 cc., 0.5 cc., 0.63 cc., 0.75 cc., 0.87 cc., 1.0 cc. and 1.5 cc. of the standard. Dilute each with distilled water to 6 cc. Add 1.2 cc. of 20 per cent trichloroacetic acid and continue as in the above procedure. These correspond respectively to 75, 100, 125, 150, 175, 200 and 300 mg. of sodium bromide per 100 cc. of serum. Over 150 mg. per 100 cc. of sample indicates the approach of bromide intoxication.

#### BROMIDES BY EXTRACTION AS BROMINE

The process is, briefly, to liberate all bromides as bromine, extract with carbon tetrachloride and compare with standards.<sup>6</sup> The method was developed for bromides in natural brines but should have a wider applicability. Traces of iodine do not interfere.

**Sample**—Make 100 cc., or other suitable volume according to concentration, alkaline with sodium carbonate and evaporate to dryness. Take up with 25 cc. of distilled water and filter into a 250 cc. volumetric flask, washing thoroughly. Add 5 cc. of 1:1 sulfuric acid and test for acidity. If not acid continue the addition until acid to Congo red paper. Dilute to volume.

**Procedure**—Pipet 25 cc. into a 50 cc. Nessler tube and add saturated chlorine-water until a distinct excess is present as shown by further additions giving no additional color. Dilute to 50 cc. Prepare a standard from standard sodium or potassium bromide solution <sup>6a</sup> by diluting to 25 cc. and treating with chlorine-water in the same way. Dilute to 50 cc.

Extract the bromine from sample and standard by shaking with 10 cc. of carbon tetrachloride solution. Separation of the solution from

<sup>5</sup> Henry Tod, *J. Mental Sci.* 79, 373-5 (1933).

<sup>6</sup> O. R. Sweeney and James R. Withrow, *J. Ind. Eng. Chem.* 9, 674 (1917).

<sup>6a</sup> See p. 544.

carbon tetrachloride is most conveniently carried out by filtration on a wet paper, later puncturing the paper to get the carbon tetrachloride solution of bromine. Better results will be obtained if filtration is carried out in a darkened room.

Compare by balancing or dilution with carbon tetrachloride to get an approximate bromide estimation. Repeat the determination with another aliquot of the sample solution using a standard or set of standards based on the first estimation. Ordinarily a single extraction will give all of the bromine, within the other errors of the method. Further refinements of the process should be possible, similar to those which have been introduced into the extraction method for iodine by J. F. McClendon.<sup>7</sup>

#### BROMIDES BY FLUORESCEIN

The reaction of bromine with fluorescein to form eosine may be used for quantitative estimation of bromides,<sup>10,11,12</sup> even in the presence of large amounts of chlorides. The greenish yellow color is altered to pink. Fluorescein paper has also been used for the estimation, evolving the gas in the general style of the Gutzeit method.<sup>13</sup> The bromine is best liberated by a suitable oxidizing agent which will liberate bromine but not chlorine. More drastic oxidation can be used and results still estimated. Iodine can be first distilled from acid solution containing ferrie alum,<sup>14</sup> then the bromine liberated by potassium permanganate. Per-nitric acid formed by reaction of nitrous acid and hydrogen peroxide has also been used as oxidizing agent.<sup>15</sup>

**Reagent—Fluorescein.** Prepare a 3.3 per cent solution of fluorescein in 0.1 *N* sodium hydroxide solution. Dilute 5 cc. of this to 1 liter as reagent.

**Buffer.** Mix 1 volume of a *N* solution of sodium acetate with 0.1 volume of *N* acetic acid. This buffer should fall in the range of pH 5.5–5.6. It can be checked with indicators.<sup>16</sup>

<sup>7</sup> See p. 553 et seq.

<sup>10</sup> H. Baubigny, *Compt. rend.* 125, 654-7 (1897).

<sup>11</sup> H. Zondek and A. Bier, *Klin. Wochenschr.* 11, 633-6 (1932).

<sup>12</sup> Fr. Hahn, *Compt. rend.* 197, 245-7 (1933).

<sup>13</sup> R. Lorenz, E. Grau and E. Bergheimer, *Z. anorg. allgem. Chem.* 136, 90-4 (1924).

<sup>14</sup> A. Labat, *Bull. soc. chim.* 9, 393-8 (1911).

<sup>15</sup> A. V. Pavlimova, *Ukrainskii Khim. Zhurnal* 5, Sci. Pt. 231 (1930).

<sup>16</sup> See pp. 677-86.



**Procedure**—Add 1 drop of the fluorescein reagent to 1 cc. of sample solution. Add 3 drops of buffer solution and 1 drop of 0.1 *N* sodium *p*-tolylchlorosulfonamate. Mix and let stand for 1 minute. Add 1 drop of 5 per cent sodium hydroxide solution containing 0.5 per cent of sodium hyposulfite. This latter addition stops the development of color. Compare with a series of standards containing 0.0001, 0.0002, 0.0004, 0.0008, 0.0013 and 0.002 mg. of bromine. For larger amounts of bromine increase the amount of fluorescein reagent.

#### BROMIDES BY PHENOL RED

Phenol red, like fluorescein, absorbs small quantities of bromine to form an indicator of the bromophenol blue type. This takes place at pH 8.7–8.8 in a borax buffer solution in the presence of calcium hypochlorite and is suitable for colorimetric estimation.<sup>17</sup> Any chloro-compound formed does not differ in color from the phenol red at pH 5.0–5.4, at which the comparison is made. The color is about constant for 0.018–0.03 mg. per cc. and above that concentration the indicator is attacked. Reducing agents including ammonia interfere. Iodides interfere because they behave like bromides.

**Reagents**—*Calcium hypochlorite*. Extract “H.T.H.”<sup>18</sup> calcium hypochlorite with water and filter. Dilute the filtrate to an oxidizing value of 0.1 *N*, within 10 per cent, by titration. For use dilute to one-tenth strength, 0.01 *N*.

*Acetate Buffer*. Dissolve 30 cc. of glacial acetic acid and 68 grams of sodium acetate trihydrate in water and dilute to 1 liter. This has a pH of 4.6–4.7. It need not be free from bromides.

**Sample**—*Removal of reducing or oxidizing agents*. Render the sample alkaline, evaporate to dryness and, if necessary, ignite. Take up with a suitable volume of water and proceed.

*Removal of iodides*. To 10–15 cc. of sample add 2 cc. of *N* sulfuric acid and 1 cc. of 0.5 *M* sodium nitrite solution for each 60 mg. of iodide present. Boil gently with stirring until the solution becomes colorless. Wash the sides of the flask with water to replace that lost by evaporation and add a few more drops of sulfuric acid and nitrite solution. If a color

<sup>17</sup> V. A. Stenger and I. M. Kolthoff, *J. Am. Chem. Soc.* 57, 831-3 (1935).

<sup>18</sup> This is a grade manufactured by the Matheson Alkali Co. having the formula  $\text{Ca}(\text{OCl})_2$ .

appears add more acid and nitrite and repeat the boiling. When the solution finally remains colorless, boil 2 minutes longer, wash the sides of the flask and cool.

*Neutralization of acid.* If the sample is not neutral add the proper amount of phenol red indicated in the procedure below. Adjust with a suitable concentration of hydrochloric or sulfuric acid, or carbonate-free sodium or potassium hydroxide solution. The end-point is a yellow color. Consider this dilution in the procedure.

**Procedure**—*Not over 0.004 mg. of bromide per cc.* Pipet 1 cc. of the sample solution into a 5 cc. vial. Add 0.05 cc. of a solution containing 10 mg. of phenol red and 1 cc. of 0.1 *N* sodium hydroxide solution per 100 cc. Add 0.2 cc. of saturated borax solution. Add 0.2 cc. of 0.01 *N* calcium hypochlorite reagent, mix and let stand for exactly 4 minutes with occasional shaking. Add 0.05 cc. of 0.1 *N* sodium arsenite solution and 0.2 cc. of acetate buffer. Compare with standards prepared in the same way. The color is yellow below 0.001 mg., reddish from 0.0015–0.002 mg. and blue-violet above 0.0025 mg. The accuracy in this range is 15 to 20 per cent.

*From 0.003–0.018 mg. of bromide per cc.* Follow the preceding procedure using a 10 cc. sample in a 20–25 cc. vial, 0.2 cc. of phenol red, 2.0 cc. of borax, 0.2 cc. of 0.1 *N* hypochlorite, 0.5 cc. of arsenite and 1.5 cc. of acetate buffer. Accuracy of about 10 per cent is obtainable.

**Standard**—Dissolve 0.0149 gram of potassium bromide in water and dilute to 1 liter. Each cc. contains 0.01 mg. of bromide.

## CHAPTER LV

### IODINE

#### IODINE BY SOLVENT EXTRACTION

IODINE present in minute amounts may be liberated by colorless oxidizing agents<sup>1,2,3</sup> and extracted with organic solvents such as chloroform or carbon tetrachloride.<sup>4,5</sup> The use of permanganate solution for oxidation has also been proposed.<sup>5a</sup> Another method is to oxidize it to iodate and use that to liberate iodine from hydriodic acid for extraction, thus increasing the sensitivity of the procedure. In such solvents it gives a pink color, which is suitable for estimation. The methods of McClendon and Remington have been developed with a fineness of detail which cannot be given in full.<sup>6</sup> The error is not over 1 per cent if 1 mg. of iodine is present. The refinement of method developed includes the use of miniature Cottrell precipitators<sup>7</sup> and other detailed combustion equipment.<sup>7,9</sup> In short, the micro analysis of iodine is not so much a problem of chemical methods as of mechanical equipment for the isolation of a minute amount of iodine from large samples.

The presence of chlorides of sodium, magnesium or calcium causes errors in the chloroform extraction. Sulfates do not affect the results in that way.<sup>10</sup>

Extreme precautions as to purity of reagents are essential.<sup>11</sup> Potas-

<sup>1</sup> Th. von Fellenberg, *Mitt. Lebensm. Hyg.* 14, 161-240 (1923); *Biochem. Z.* 152, 116 (1924).

<sup>2</sup> G. Pfeiffer, *Biochem. Z.* 201, 298-304 (1929).

<sup>3</sup> Eric N. Allott, James A. Dauphinee and Wm. H. Hurtley, *Biochem. J.* 26, 1665-71 (1932).

<sup>4</sup> P. A. Meerburg, *Z. physik. Chem.* 130, 105-8 (1927).

<sup>5</sup> J. F. McClendon, *J. Biol. Chem.* 60, 288-99 (1924).

<sup>5a</sup> A. C. Bose and K. N. Bagchi, *Analyst* 60, 80-2 (1935).

<sup>6</sup> Roe E. Remington, J. F. McClendon and Harry von Kolnitz, *J. Am. Chem. Soc.* 53, 1245-9 (1931).

<sup>7</sup> George M. Karns, *Ind. Eng. Chem., Anal. Ed.* 4, 299-300, 375-7 (1932).

<sup>9</sup> Harry von Kolnitz and Roe E. Remington, *Ind. Eng. Chem., Anal. Ed.* 5, 38-40 (1933).

<sup>10</sup> K. L. Malyarov and V. B. Matskievich, *Mikrochemie* 13, 85-90 (1933).

<sup>11</sup> R. G. Turner and Mina Z. Weeks, *J. Am. Chem. Soc.* 55, 254-8 (1933).

sium carbonate must be free from iodine. Sulfuric acid must not contain over 0.00008 per cent of iron and be free from oxidizable foreign substances. Aldehydes must be removed from alcohol by a reduction procedure such as the following.<sup>12</sup> Add 0.5 gram of iodine crystals to 500 cc. of absolute alcohol. Let stand for 24 hours and distil. Discard the first 25 cc. and leave 50 cc. in the flask. Shake the distillate with 100 grams of zinc until it is no longer yellow. Distil as previously described. Add 100 grams of granulated zinc and shake for 15 minutes. Distil as before.

**Sample—Water.**<sup>13</sup> Evaporate 3 to 6 liters of water containing a few drops of 0.01 per cent phenolphthalein solution and about 10 cc. of 0.6 per cent potassium carbonate solution, to about 150 cc. Keep the solution alkaline by means of added potassium carbonate solution if necessary. Filter to remove calcium carbonate and iron and evaporate the filtrate nearly to dryness in platinum. Extract the residue of moist salts with 3 portions of 80 to 95 per cent alcohol. Evaporate the residue to dryness and ignite carefully. Moisten with a few drops of 0.6 per cent potassium carbonate solution and again extract with alcohol. Evaporate the combined alcoholic extracts to dryness. Ignite very carefully and take up in 1 cc. of water. Add 1 drop of 1:1 sulfuric acid.

**Water. Alternative Method.**<sup>14</sup> If the iodine content of water is low, as much as 100 liters may be necessary as sample. The solution must remain alkaline to phenol red during the evaporation to prevent loss of iodine. For this large-scale evaporation prepare a clean barrel with a 1 inch pipe as exit tube to feed the pan used for evaporation. Add sodium carbonate so that the sample is alkaline. Evaporate rapidly in a large dish pan containing 2 grams of sodium bicarbonate, adding more sample water from time to time, to a volume of 1 liter. Filter and evaporate the filtrate to dryness. Powder and place in a silica, nickel or iron boat in a silica tube. Heat to a dull red and lead the exit gases through 10 cc. of 10 per cent sodium hydroxide solution. Pass oxygen through the tube during the heating, which should be as brief as possible with complete combustion of the organic matter.

Evaporate the absorption solution and rinsings of the combustion tube to dryness. Mix with the ash, and powder. Add 15 cc. of water and grind in a mortar. Filter and take an aliquot of 7.5 cc. Neutralize

<sup>12</sup> A. Castille and V. Henri, *Bull. soc. chim. biol.* **6**, 299-302 (1924).

<sup>13</sup> P. A. Meerburg, *Z. physik. Chem.* **130**, 105-8 (1927).

<sup>14</sup> J. F. McClendon, *J. Biol. Chem.* **60**, 288-99 (1924).

with concentrated hydrochloric acid, add 1 drop in excess and dilute to 10 cc.

*Vegetables and other Foodstuffs.*<sup>15</sup> A sketch of the apparatus is shown in Figure 80. A steel tube is prepared. One end is open and water cooled. The other has a screw arrangement like an "Alemite" gun. The sample is made into a dried stick to fit the tube. This is slowly advanced by operation of the screw into a silica furnace in which it is burned in oxygen. The exit gases are washed in four Milligan wash bottles filled with 0.25 per cent sodium hydroxide solution and cooled

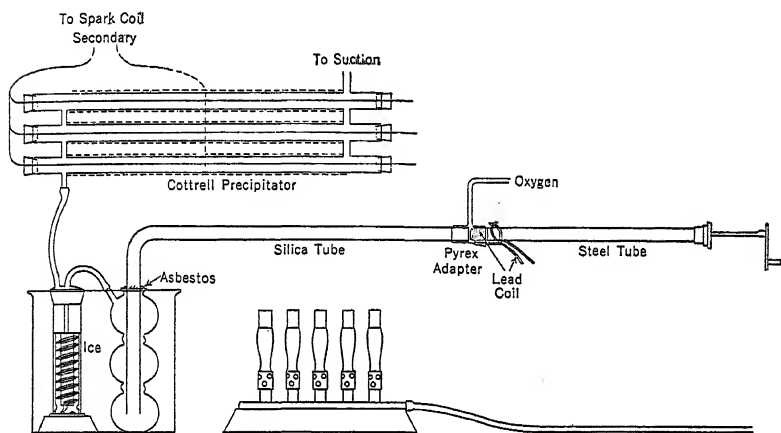


FIG. 80

Apparatus for Ashing Samples for Iodine Determination

with ice. The gas then passes through a miniature Cottrell precipitator. As a slightly less efficient set-up, four additional wash bottles and a 25 × 500 mm. test tube packed with glass wool may be used in place of the precipitator.

In operation pass the gas at the rate of 1.25 cubic feet per minute. To start, ignite the dried stick and advance at such a rate that no soot or tarry matter is formed.

Grind the residual ash in a ball mill with 0.25 per cent sodium hydroxide solution and decant. Evaporate this extract, the contents of the absorption bottles and the residue from the precipitator in a large beaker. When nearly dry transfer to an evaporating dish and take to dryness. Ignite at a low temperature in a nickel boat in a combustion

<sup>15</sup> J. F. McClendon and Roe E. Remington, *J. Am. Chem. Soc.* 51, 394-9 (1929).

tube and wash the exit gas with 0.01 *N* sodium hydroxide solution using a side-arm test tube. During this treatment, do not heat to a temperature at which the residue will fuse.

Place the ash in a small beaker and add the absorption solution. Then add, drop by drop, a mixture of 9 parts of syrupy phosphoric acid and 1 part of 0.1 *N* sulfurous acid until effervescence ceases. Boil gently for 5 minutes to expel sulfur dioxide. If the solution does not readily change bromophenol blue paper to yellow, add concentrated sulfuric acid drop by drop until it does. Dilute to 10 cc. with distilled water.

*Foodstuffs. Alternative Method.*<sup>15</sup> If the fat content of the sample is low prepare in the form of compressed tablets. Burn these in an iron boat in place of using the injector described. The balance of the method is the same, including washing of the gas.

*Foodstuffs. Alternative Method.*<sup>15</sup> For cereal grains and other acid ash samples moisten the sample with 50 cc. of 2 per cent sodium carbonate solution. This will result in a high fusing-point alkaline ash. Dry and grind to coarse granules. Heat in an evaporating dish over a small flame until it begins to smolder. Withdraw the heat and let burn without flame. Ignite again if necessary, giving a black char at last. Heat in a muffle furnace at not over 450° to give a light grey ash. A white ash is impractical as 12 to 15 hours will be required for the grey color. Extract and proceed as before. By this method a check within 5 per cent was obtained on 0.1 mg. of iodine added. A modification<sup>18</sup> calls for ashing below 400°.

*Foodstuffs. Alternative Method.*<sup>18a</sup> A separate procedure for ashing in a combustion furnace has been described. The equipment is shown in Figure 81. Potassium carbonate solution is used for absorption; for this equipment sodium bisulfite has been found unsatisfactory.

Weigh 50 grams of air-dried sample into a porcelain dish and mix with 10 grams of finely pulverized calcium oxide and 10 grams of finely pulverized copper oxide. Transfer to alundum boats and place in a combustion furnace. Connect the absorption bottles on the left with a suction pump. Turn on the electric furnace and when the tube 3 attains a red heat, start the suction pump and light the first gas burner at the left of the series, 9. The heat from this should set the contents of the first boat on fire. The combustion is maintained at a regular rate by igniting other burners of the gas furnace. Any unburned vapors from

<sup>15</sup> J. S. McHargue, *J. Assoc. Official Agr. Chem.* 14, 222-3 (1931).

<sup>18a</sup> J. S. McHargue, D. W. Young and R. K. Calfee, *Ind. Eng. Chem., Anal. Ed.* 6, 318-9 (1934).

the sample are drawn over the catalyst 4 and completely burned. The iodine vapors are absorbed in the gas wash bottles.

When combustion is complete turn off the heat and cool with a current of air drawn through the system. Turn off the suction and remove the boats. Digest the ash with hot distilled water. Filter and combine the filtrate with the potassium carbonate solution from the absorption flasks. Evaporate to dryness and take up with a minimum volume of water so that the solution is nearly saturated with potassium carbonate. Transfer

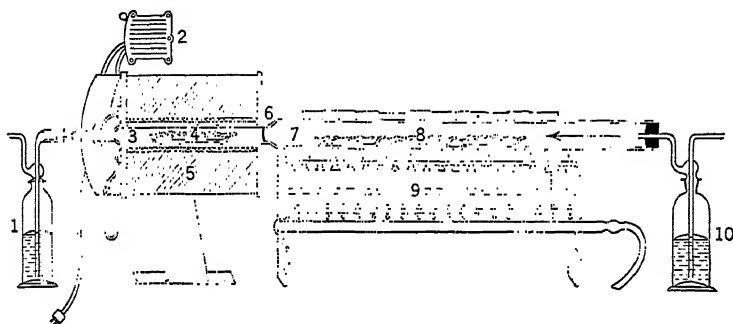


FIG. 81

Diagram of Apparatus for Combustion of Samples of Vegetables for Iodine Determination

1. Absorption bottle containing 5 per cent potassium carbonate solution. (2 bottles used.)
2. Rheostat.
3. Silica catalyst tube.
4. Platinized asbestos catalyst.
5. Electric tube furnace.
6. Asbestos cement seal.
7. Silica combustion tube.
8. Alumina boats containing samples.
9. Gas combustion furnace.
10. Wash bottle containing 10 per cent potassium hydroxide solution.

to a separatory funnel and add sufficient 95 per cent alcohol to form 2 layers. Shake vigorously for about 10 minutes and separate the alcoholic layer. Repeat this extraction 3 times. Combine the alcoholic extracts containing the iodide and evaporate to dryness at a rate to avoid spattering. Dissolve the residue in a few drops of water and filter.

Add 3 cc. of a saturated solution of sulfurous acid to the sample in a separatory funnel and make it distinctly acid with 1:4 sulfuric acid. Stopper and shake for 1 minute to insure complete reduction to iodide. Transfer to a beaker and heat gently to drive off sulfur dioxide. Use this solution as sample, diluting if desirable.

*Thyroid.*<sup>19</sup> Thyroid free from fat may be mixed with potatoes of known iodine content and analyzed by the preceding method. A modified method designed solely for organic matter high in iodine is preferable.

Cut the thyroid into small bits. Dry at 100° for 24 hours, obtaining a rough moisture determination at the same time. Grind the resulting glassy mass and again determine moisture at 100°. Extract the fat with anhydrous ether in an apparatus in which the sample is surrounded by vapors of the boiling solvent. The Bailey-Walker apparatus is suitable. Weigh 0.1 gram of powdered extract into a 2 × 5 × 1 cm. nickel boat with one end cut away. Mix with about 2 volumes of freshly ignited calcium oxide, using a platinum wire. Cover completely with a thin layer of lime.

The apparatus for combustion is a modification of that for a previous method.<sup>19a</sup> The combustion tube of Pyrex is 210 × 24 mm. The outlet tube is 155 × 5 mm. Replace the absorption bulbs by 150 × 15 mm. test tubes containing 0.1 per cent sodium sulfite solution. Use 2 of these in parallel, splitting the gas stream with a Y tube. As an inlet use 3 mm. capillary tubes further drawn out to a fine tip. Cool the tubes in ice water. Unite the gas stream with another Y after passing these and pass through a 235 × 10 mm. vertical soda-lime tube. Put a glass filter disc in the bottom of this and fill with glass wool moistened with 0.1 per cent sodium sulfite solution. Connect the soda-lime tube to a suction flask connected to a pump. Fit the rubber tube connecting to the suction flask with a clamp to regulate the rate at which gas is drawn through. For heating join two blowpipes or use a special apparatus.<sup>20</sup> The bent end should be about 1 cm. long. Through one pass illuminating gas and through the other pass oxygen.

Insert the boat in the combustion tube about 4 mm. from the inner end. Regulate the flow of air. Ignite the torch to give a nonluminous flame, oxygen being in excess. Smoked glasses will assist in observing the combustion. Heat, starting at the far end of the boat. After the first burning let the boat cool and mix the contents with a platinum wire. Again ignite in the tube to burn out any black spots and repeat if necessary. The absorbers should be clear and colorless. Fat must be absent from the sample as it will distil onto the tube.

Rinse receivers and combustion tube into a 250 cc. beaker. Add the

---

<sup>19</sup> Roe E. Remington, J. F. McClendon, Harry von Kolnitz and Bartow Culp, *J. Am. Chem. Soc.* 52, 980-5 (1930).

<sup>19a</sup> See p. 553.

<sup>20</sup> Hoke, Inc., New York, N. Y.



boat and residue. The tube should first be rinsed with alcohol, then with water and the rinsings added. The washings should not exceed 225 cc.

Evaporate to about 50 cc. Centrifuge to remove insoluble lime and evaporate to a small volume. Transfer to a nickel or platinum boat and evaporate to dryness.

Sprinkle about 0.5 gram of powdered sodium hydroxide over the residue and place in a combustion tube such as was used for foodstuffs. Heat by a flame underneath until completely fused. Draw air through and use only one absorption bulb. This is no more trouble than heating in an open dish and avoids overheating and loss of iodine. Careful heating without this apparatus can be substituted.

Allow the flux to cool, and dissolve in 10 cc. of water. Transfer to a 50 cc. beaker. To this add the rinsings of the tube and the contents of the absorption bulb. Add about 5 mg. of sodium azide.<sup>21</sup> This removes any nitrites formed by combustion of proteins. Make just acid to bromophenol blue paper with syrupy phosphoric acid by testing small drops on the paper outside of the solution. Add 5 to 10 drops excess of the phosphoric acid and 5 drops of 8 per cent sodium sulfite solution.

Heat until the odor of hydrazoic acid disappears. Transfer to a 50 cc. volumetric flask and dilute to volume. Transfer 10 cc. to a separatory funnel for the determination. If the iodine content is very low evaporate to 5-8 cc. before acidifying, dilute to 10 cc. when acid and extract the entire sample. Checks with the gravimetric method of Kendall are good.

*Fat.*<sup>22</sup> Melt the fat and if necessary filter to free from water and suspended matter. Place in a large atomizer or lacquer spray. Spray into a heated silica tube having a 1 inch bore, 2 feet long with an elbow 1 foot long, using oxygen. The heating equipment is similar to that of previous methods. It is essential that the tube be very hot and in particular that a point just in front of the spray be kept particularly hot with a small blast flame or auxiliary heating coil. Place a platinum foil or spiral of heavy platinum wire in the tube as catalyst. Surround the end of the tube near the nozzle with a pad of moist asbestos cooled by dropping water. This also cools the nozzle and prevents carbonizing on it.

As absorbents use 10 Milligan wash bottles in parallel, each containing 0.25 gram of sodium sulfite in water. This insures reduction to iodide. Follow these with a tower packed with glass wool. Draw the gases

<sup>21</sup> J. F. Reith, Dissertation, Univ. of Utrecht, 1929.

<sup>22</sup> J. F. McClendon, Roe E. Remington, Harry von Kolnitz and Redding Rufe, *J. Am. Chem. Soc.* 52, 541-9 (1930).

through the furnace and absorbers at 2 cubic feet per minute by use of a rotary air pump. In operation, if the ratio of oxygen to fat is too high, the fatty acids will distil over instead of burning, and if too low, soot will form. As much as 500 grams of sample may be necessary. This is determined by weighing the atomizer before and after the combustion. Soot and tarry products can be entirely avoided.

When combustion is complete, transfer the contents of the absorbers and the rinsings of the apparatus to a beaker. Evaporate to small volume, transfer to a nickel boat and evaporate to dryness. Ignite in a Pyrex tube. Use 5 cc. of water and a few mg. of sodium sulfite in a side-arm test tube to absorb volatilized iodine.

Place the boat in a very small test tube with the absorbing solution and rinsings of the tube for the ash to dissolve. Centrifuge from insoluble material and decant into a 30 cc. beaker. Add 5 drops of a saturated solution of sulfur dioxide to reduce iodate and 5 mg. of sodium azide to reduce nitrites. Neutralize with syrupy phosphoric acid and add 4 drops in excess. If the odor of hydrazoic acid is not present add more. Boil to drive off excess hydrazoic acid and sulfur dioxide. Transfer to a separatory funnel and dilute to 10 cc.

By this method butter fat from various parts of the United States was shown to vary in iodine content from 4 to 78 parts per billion.

*Oil Seeds, Nuts and Dry Milk.* Pack in a sausage casing and handle similarly to foodstuffs. An auxiliary oxygen feed at the opening of the tube is necessary. Ceria or thoria will catalyze combustion of milk.

*Eggs.*<sup>23</sup> Place the liquid contents of the eggs in a flask. Add an equal volume of 95 per cent alcohol and 10 grams of potassium hydroxide for each egg. Reflux for 24 hours. No foaming or bumping occurs and the product is a dark brown liquid, nearly free of solids. This converts organic iodine into iodide ion. Transfer an amount of the liquid equivalent to 1 egg to a 500 cc. nickel crucible, or, if that is not available, to a beaker. Evaporate to dryness. Place in a muffle furnace and ash for 4 hours at 600°

Extract the ash with 50 cc. of hot water. Filter and wash the residue with hot water. Acidify the filtrate and washings with 1:2 sulfuric acid until acid to methyl red. Add 5 drops more of acid.

*Urine.* Neutralize with lime water and add about 5 cc. of extract of jack bean meal. The urease present hydrolyzes urea. Add 5 grams of lime and evaporate to a small volume. When concentrated to small volume transfer to a nickel boat. Add ceria or thoria and sulfur.

---

<sup>23</sup> H. J. Almquist and J. W. Givens, *Ind. Eng. Chem., Anal. Ed.* 5, 254 (1933).

Evaporate to dryness and ignite in a heated Pyrex tube furnace with an oxy-gas torch or electrically heated platinum spiral.

Grind the ash for 1 hour with 100 cc. of 95 per cent alcohol in a ball mill. Follow with 100 cc. of 80 per cent alcohol for 1 hour. Combine the extracts, centrifuge and decant. Evaporate the extracts, absorbing solutions and washings of the tube in porcelain to a small volume and if necessary centrifuge again. Transfer to a nickel boat, evaporate to dryness, ignite in a smaller Pyrex tube and proceed as for iodine in fat.

*Urine.*<sup>24</sup> *Alternative Method.* Evaporate 2.5–4 liters of urine with 10 grams of sodium hydroxide to a thick syrup. Transfer to a small iron pan and add 20 grams more of sodium hydroxide. Heat slowly until fumes are evolved. Let cool and grind the clinker in a mill. Let stand over night in a beaker with 200 cc. of alcohol. Decant through a filter and wash by decantation several times with alcohol. Transfer to the filter and wash several times with alcohol.

Evaporate the filtrate until the major portion of the alcohol has been driven off. Add 3 grams of sodium hydroxide and evaporate to dryness in a nickel dish. Heat until volatile matter is driven off and the residue charred. Extract with 25 cc. of water and filter. Evaporate to 5 cc. and add 2 drops of 0.1 *N* arsenious acid. Add 1:3 sulfuric acid until distinctly acid and transfer to a separatory funnel.

The recovery of iodine added to urine was about 75 per cent by this method.

*Urine. Inorganic Iodine.*<sup>25</sup> Acidify 5 cc. with a drop of 1:1 sulfuric acid. If iodine is in the organic form, direct determination of the inorganic iodine may give a brown color in the solvent.

*Blood.*<sup>26</sup> Mix 5 cc. of oxalated blood with 1 cc. of 50 per cent potassium hydroxide solution in a porcelain crucible. Concentrate to a thick paste on the water bath and heat cautiously at 400–500°. The material should char completely in 40 minutes to give a dry black powder. Transfer this quantitatively to a porcelain boat and burn in a combustion tube in a stream of oxygen. Proceed cautiously by slow admission of oxygen. The combustion should require only 5 to 6 minutes. No significant loss of iodine occurs.

Extract the residue with 5 cc. of 95 per cent alcohol in a micro extractor for about 20 minutes. The alcoholic extract should be clear and colorless. Transfer the extract quantitatively to a gold dish and rinse the

<sup>24</sup> H. W. Clark and George O. Adams, *Am. J. Pub. Health* 19, 900 (1929).

<sup>25</sup> W. Autenrieth and A. Funk, *Münch. med. Wochschr.* 59, 2736 (1912).

<sup>26</sup> A. Wladyslaw Elmer, *Biochem. Z.* 248, 163-7 (1932).

apparatus 3 times with 1 cc. portions of water. Render the solution alkaline with 5 drops of 50 per cent potassium hydroxide solution. Evaporate to dryness on a water bath and ignite the residue over a free flame until it is white. If necessary, moisten with water and repeat the drying and ignition.

Moisten the residue to a paste with 2 cc. of absolute alcohol and a drop of water. If not pasty when worked with an agate pestle, excess alkalinity is not present, iodine has been lost and the estimation must be repeated. Transfer the alcoholic extract to a platinum dish. Wash the residual salt with 4 more 2 cc. portions of absolute alcohol. Add 5 cc. of water and evaporate to dryness on a water bath. Dissolve the residue in 1 cc. of water and transfer to a flask. Rinse the dish with four 0.2 cc. portions of water. Proceed as for iodine in fat.

In the original method the iodine is oxidized with bromine to iodate and liberates iodine, which is estimated by its color with starch.

*Soil or Rock.*<sup>27</sup> Iodine present in soil or rock is volatilized on heating. A furnace capable of heating a 100 gram sample in a closed tube is required.

Place 25 to 100 grams of soil or small pieces of rock in a boat in the furnace. Connect the exit of the combustion tube with 3 gas bottles; loosely stopper the inlet end with an alundum crucible. Fill the wash bottles with a 5 per cent aqueous solution of potassium carbonate. Connect the last wash bottle to a suction pump.

Bring the furnace up to 1100° in about 2 hours and continue to heat for 2 hours, drawing air through at a slow rate. Rinse the contents of the wash bottles into a porcelain dish and evaporate to dryness. Take up the residue with 10 cc. of water and filter into a small porcelain dish. Evaporate to dryness and ignite gently to destroy organic matter. When cool dissolve in 0.2 cc. of water and filter into a small separatory funnel. Wash the filter with 0.1 cc. of water, then with absolute alcohol. Add absolute alcohol until two layers are formed. Shake and separate the alcohol layer. Repeat twice. Evaporate the alcoholic filtrates to dryness and take up with 1 cc. of water. Acidify with 1 drop of 1:1 sulfuric acid.

*Miscellaneous Samples by Distillation.*<sup>28</sup> To avoid loss by decom-

---

<sup>27</sup> J. S. McHargue, D. W. Young and W. R. Roy, *Ind. Eng. Chem., Anal. Ed.* 4, 214-6 (1932).

<sup>28</sup> G. Pfeiffer, *Biochem. Z.* 195, 128 (1928); *Ibid.* 201, 298 (1928); *Ibid.* 210, 231 (1929); *Ibid.* 215, 128 (1929); *Ibid.* 228, 148 (1930); *Ibid.* 231, 244 (1931); *Ibid.* 241, 280 (1931); *Ibid.* 256, 214 (1932).

position and volatilization of potassium iodide at  $650^{\circ}$ , which may occur in combustion methods, hot acid may be used for decomposition and the resulting iodine or hydriodic acid distilled and absorbed.

Liquid samples are made alkaline and concentrated to small volume or dried. Protein or calcium sulfate may be added as a carrier. Liquids such as blood, milk, tissue fluids, etc. are used directly if high in iodine. Mix eggs thoroughly and use a 15 gram sample. For egg white alone, use 20 grams and for yolks alone use 10 grams. Cut up or grind plant or animal products. Add oils and fats slowly in small portions to avoid fatty acids distilling over.

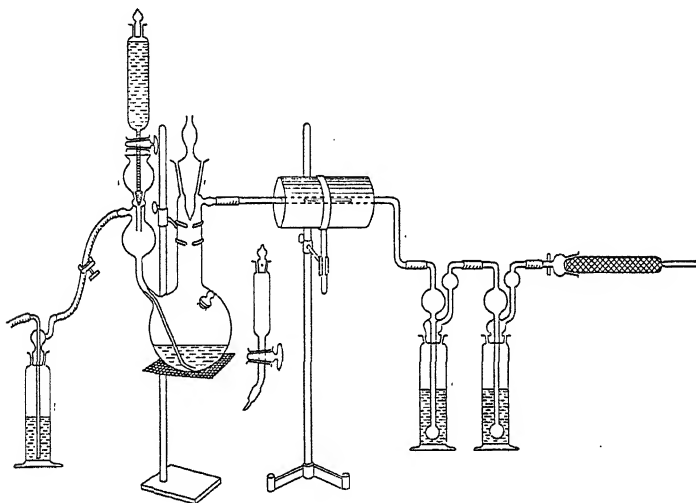


FIG. 82

Apparatus for Distillation of Iodine from Samples

The equipment is shown in Figure 82. A 500 cc. flask is used for 3 to 5 grams of dry substance, or a 1 liter flask for a sample up to 10 grams. A dropping funnel provides for addition of acid and later of hydrogen peroxide. Pass the air used as carrier through a wash bottle containing potassium hydroxide solution to remove iodine. From the distillation flask lead it through two absorption cylinders. Each is half filled with glass beads and contains 3 per cent potassium hydroxide solution. A third absorption tube is filled with broken pieces of crockery wet with 10 per cent potassium hydroxide solution. When there is a possibility of fatty acids distilling over, insert a quartz tube with a

platinum contact surface to decompose them. This is heated to 800–900°.

Add 100–150 cc. of concentrated sulfuric acid to the sample in the flask. If necessary to reduce the rate of digestion, 20–30 cc. of water may be added. Start the flow of air and heat the digestion flask gradually up to 200°. If digestion is slow to start, add 5 to 10 drops of 30 per cent hydrogen peroxide before heating. Add it a few drops at a time during the heating.

Unite the liquids resulting from condensation. Evaporate them to dryness and extract iodine with 50 cc. of alcohol. Decant and extract with 2 additional 10 cc. portions of alcohol, being sure to break up all lumps. Render this solution alkaline with 5 drops of 10 per cent potassium hydroxide solution and evaporate to dryness. Gently ignite to destroy organic matter. Add 2 drops of saturated sulfurous acid solution and 5 cc. of 1:9 sulfuric acid. Filter and wash the filter with 2 cc. of water. Use this solution as sample.

**Purification of Solvents**—To purify carbon tetrachloride or chloroform for use with this method add bromine-water until the color persists. Remove excess bromine by several extractions with 0.1 per cent sodium carbonate solution. Wash with water, filter, distil and reject the first fraction.

**Procedure**—*Mild Oxidation*. To the sample add 10 per cent of its volume of carbon tetrachloride or chloroform and shake. A pink color at this stage indicates the presence of iodate as well as iodide. If present, add 1 drop of 0.1 *N* arsenious acid and let stand 10 minutes to reduce. Add 1 drop of 10 per cent potassium nitrite, and shake for 2 minutes to extract iodine. Centrifuge to clarify the solvent solution of iodine.

To insure the complete liberation of iodine withdraw 0.1 cc. of the aqueous layer, add 1 drop of chloroform or carbon tetrachloride and a small crystal of potassium iodide. Unless this solution becomes pink add a drop of concentrated sulfuric acid to the main solution and repeat the extraction.

Withdraw the solvent solution and compare in a colorimeter with a solution of standard similarly prepared. As an alternative, compare with a solution of iodine in the same solvent, containing 0.1 mg. of iodine per cc., by the dilution method. To compensate for incomplete extraction multiply the result so obtained by 1.118.

*Drastic Oxidation*.<sup>23</sup> Add saturated bromine-water to the acid sample until a permanent and definite yellow color is obtained. Boil until excess

bromine is removed and the solution has been concentrated to a small volume. Cool and transfer the iodic acid solution to a small separatory funnel. Wash any crystals which have separated and add the washings.

Add a crystal of potassium iodide. Extract the liberated iodine with five 1 cc. portions of pure carbon tetrachloride. Compare the combined extracts with a standard.

**Standards**—Dissolve 0.1308 gram of potassium iodide in water and dilute to 1 liter. Dilute 100 cc. of this to 1 liter. Each cc. of the latter corresponds to 0.01 mg. of iodine.

For a series of standards measure out 0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 cc. of this solution. Dilute each to the same volume as the sample and treat exactly as the sample. Extract with the same solvent.

#### IODINE AS SILVER IODIDE BY CONVERSION TO SILVER SULFIDE

When the combined chlorides and iodides are precipitated by silver nitrate, the silver chloride can be dissolved with ammonia with practically no effect on the silver iodide. The silver iodide is then dissolved in potassium cyanide and converted to silver sulfide for indirect estimation.<sup>30</sup> The method, which was developed for urine, is accurate within 3 per cent.

**Sample**—To estimate the volume of urine to be taken, put 0.5 cc. of 0.13 per cent potassium iodide solution into a test tube, and 0.5 cc. of urine into a second test tube. Add to each 2.5 cc. of water and 3 drops of fuming nitric acid. Dilute to about 10 cc. with chloroform. Shake the tubes. If the urine tube is much lighter in color add further portions of 0.5 cc. of urine until an approximate match is obtained. Take 10 times the amount used in this test, for the quantitative determination.

**Procedure**—Divide the sample between 4 centrifuge tubes. Add to each the same amount of water and 1 cc. of 5 *N* nitric acid, then 2 cc. of silver nitrate solution containing 50 grams per 100 cc. of solution. Stir, and centrifuge until the supernatant liquid remains clear on the addition of a drop of silver nitrate solution. Pour off the supernatant liquid and wash the precipitates with water 3 times by centrifuging and decanting off the wash water.

To each of the tubes add about 0.5 cc. of 10 per cent ammonium

<sup>30</sup> S. Yoshimatsu and H. Sakurada, *Tôhoku J. Exptl. Med.* 8, 107-12 (1926).

hydroxide, stir and centrifuge. Pour off the supernatant liquid and wash the iodide precipitates 3 times with distilled water. Dissolve the precipitate in each tube in 1 cc. of a 5 per cent potassium cyanide solution. Transfer the contents of all the tubes to a 50 cc. volumetric flask. Into a second 50 cc. flask put 10 cc. of a standard silver nitrate solution containing 0.6690 gram per liter. Each cc. is equivalent to 0.5 mg. of iodine. To this flask add 4 cc. of a 5 per cent potassium cyanide solution. Mix and add to each flask 0.5 cc. of a 10 per cent gelatin solution and 2 cc. of a 10 per cent sodium sulfide solution. The brown color develops immediately. After 1 minute dilute to volume and compare in a colorimeter.

#### IODINE BY PALLADOUS CHLORIDE

The light brown to red-brown color of palladous iodide may be used for the determination of iodides.<sup>31</sup> By reduction of iodates they may also be determined. The method is applicable to organic matter and has been applied to thyroid extract. Halogens or oxidized halogen compounds in the reagents must be avoided.

**Sample**—Mix 0.5 gram of dry thyroid in a nickel crucible with 8 grams of an equimolecular mixture of sodium carbonate, potassium carbonate and potassium nitrate. Cover with an additional 5 grams of the mixture. Adjust a cover on the crucible so as to leave a small opening for waste gases. Heat in a muffle furnace<sup>32</sup> until the edges just melt and the carbon is oxidized. This will usually require about 10 minutes. Let cool and add about 10 cc. of water, rinsing the cover. Dissolve on a hot plate and pour through a funnel into a 200 cc. Erlenmeyer flask. Wash well, but using as little water as possible. Add 2 cc. of a 10 per cent solution of sodium bisulfite to reduce any iodate. Make faintly acid to litmus with 85 per cent phosphoric acid. Let stand a few minutes and make alkaline with sodium carbonate.

With a funnel in the neck of the flask evaporate until the salts just remain in solution when cold. On cooling add 20 cc. of acetone, rinsing the funnel and sides of the flask. Shake until the salts form a semisolid layer in the bottom. Add about 40 cc. of absolute alcohol in 5 cc. portions, stirring to dissolve the iodides. Decant through a filter into a comparison tube if the amount of iodine is under 1 mg. Otherwise decant into a volumetric flask, dilute with alcohol and remove an aliquot.

<sup>31</sup> R. B. Krauss, *J. Biol. Chem.* 22, 151-7 (1915).

<sup>32</sup> R. B. Krauss, *J. Am. Chem. Soc.* 36, 962 (1914).



**Procedure**—Add drop by drop until the color no longer deepens, a solution containing 0.5 mg. of palladous chloride per cc., in 0.1 *N* hydrochloric acid. Each cc. is sufficient for about 0.75 mg. of iodine. Two cc. diluted to 100 cc. should show no appreciable color. Dilute with water to 100 cc. Measure 1 cc. of a standard potassium iodide solution containing 0.1308 gram per liter. This is equivalent to 0.1 mg. of iodine per cc. Place in a 100 cc. comparison tube. Add 10 cc. of acetone, 10 cc. of absolute alcohol, and 2 cc. of palladous chloride reagent. Dilute to 100 cc. and compare after 5 minutes.

The color develops best in concentrated solution. The palladous chloride should therefore be added before dilution. Comparison with a series of standards is less accurate than balancing.

#### IODINE IN THE PRESENCE OF BROMINE BY CARBON BISULFIDE EXTRACTION

Iodine may be determined by means of the violet color of its solution in carbon bisulfide.<sup>33</sup> Chlorine does not interfere, nor bromine if its concentration is less than 5 times that of the iodine. If greater amounts of bromine are present the error can be corrected by suitable manipulation. The temperature should be kept low to avoid loss of iodine. The method is applicable to amounts of iodine between 0.02 and 0.5 mg. It should be possible to use other solvents such as carbon tetrachloride and chloroform.

**Procedure**—Into a graduated cylinder of 100 cc. capacity, fitted with a glass stopper, pour 50 cc. of sample solution. Add 4 cc. of a 10 per cent solution of sodium bicarbonate, shake and add a 2.5 per cent solution of potassium permanganate to the appearance of a rose color, then a further 5 cc. of permanganate solution. Cool the cylinder to 10° and add 2 cc. of carbon bisulfide. Add 5 cc. of concentrated sulfuric acid dropwise, to avoid heating. Shake, then remove the permanganate color by careful addition of hydrogen peroxide. Shake and compare the color in the solvent layer with a standard solution of potassium iodide treated similarly.

This procedure liberates all the combined halogens, chlorine, bromine and iodine. The treatment with permanganate may be replaced by acidifying the solution with concentrated sulfuric acid saturated with nitrogen pentoxide.<sup>34</sup>

<sup>33</sup> J. Dubief, *Ann. fals.* 16, 80 (1923).

<sup>34</sup> L. C. Case, *Chemist-Analyst* 20, 9 (1931).

If the weight of bromine is more than 5 times that of iodine, iodine bromide is formed. This is shown by a yellow color developed in the carbon bisulfide layer immediately after decolorization of the permanganate. To prevent this add a drop of 0.1 *N* potassium thiocyanate solution and shake. Continue this until the yellow color completely disappears, avoiding an excess, as this would decolorize the iodine also. By careful manipulation 1 part of iodine to 2000 parts of bromine can be estimated.

If the iodine color is not suitable for comparison repeat with a larger or smaller sample.

#### IODIDES BY BROMINE-WATER

Relatively large concentrations of iodides may be determined by the brown color of free iodine liberated in acid solution by a few drops of bromine-water. Bromides and chlorides do not interfere.<sup>35</sup>

With lesser amounts the iodide is oxidized to iodate by boiling with excess bromine-water and thus 6 times the original amount of iodine is liberated from potassium iodide in acid solution by the reaction  $\text{HIO}_3 + 5\text{HI} \rightarrow 3\text{H}_2\text{O} + 3\text{I}_2$ .<sup>36</sup> This may be estimated to 0.0005–0.005 mg. in the sample by its reaction with starch paste.

**Starch Solution**—Mix 0.5 gram of soluble potato starch with 2.5 cc. of water and work to a thin paste. Pour this gradually with stirring into 200 cc. of water. Heat to boiling and boil for 15 minutes, stirring constantly. When cool add 0.25 gram of salicylic acid and stir until dissolved. Prepare fresh every 2 weeks. It should be clear and require no filtering.

**Procedure**—*Direct Liberation by Bromine-Water.*<sup>35</sup> Prepare bromine-water by dissolving 2 cc. of bromine in 200 cc. of concentrated hydrochloric acid. Dilute 20 cc. of this with water to 1 liter for use. Take 50 cc. of sample, suitably diluted if necessary, in a comparison tube. Add 10 cc. of 1:1 sulfuric acid and bromine-water dropwise until a maximum color is produced. Compare with 50 cc. of standard from which the color has been similarly liberated.

*Oxidation with Bromine-Water.*<sup>36</sup> Place the sample in a Pyrex test tube. Dilute to 2 cc. Add 2 drops of 2 *N* sulfuric acid and 3 drops of freshly saturated bromine-water. Let stand for one-half minute and

<sup>35</sup> W. J. Dilbden and L. H. Cooper, *Analyst* 35, 159-61 (1910).

<sup>36</sup> R. G. Turner, *J. Am. Chem. Soc.* 52, 2768-73 (1930); *J. Biol. Chem.* 88, 497-511 (1930).

boil off excess bromine over a micro burner. Cautiously evaporate to 0.5 cc. While hot add 1 drop of 1 per cent salicylic acid solution and cool in water. Treat 1 cc. of potassium iodide of which each cc. is equivalent to 0.001 mg. of iodine in the same way.

Add 5 drops of starch solution to sample and standard. Add 3 drops of 1 per cent potassium iodide solution to each and dilute to 1 cc. Compare in a micro colorimeter after suitable dilution. The color is stable for 1 hour and increases slightly in 16 hours. The color is not affected by temperature changes in the range of 10–60°. The method has proved its worth in 3 years of clinical use.<sup>40</sup>

**Standard**—Dissolve 0.1308 gram of potassium iodide in water and dilute to 1 liter. Each cc. corresponds to 0.1 mg. of iodine. As more dilute solutions dilute 10 cc. to 100 cc., which will then contain 0.01 mg. per cc., or to 1 liter when each cc. will contain 0.001 mg.

#### IODIDES AND IODATES BY *o*-TOLIDINE

In neutral solution iodine gives with *o*-tolidine a blue-green color similar to that for chlorine. Iodides and iodates are also determined by this color reaction after reduction of the iodates to iodides, and oxidation of the iodides to free iodine.<sup>41</sup> Nitrites give the same color reaction and must be changed to nitrates which do not interfere. Smaller amounts of chlorine or bromine than 1500 p.p.m. do not affect the color under the conditions of the method. Greater amounts lead to high results. Salts of iron, copper, mercury, etc. must be removed, as they give precipitates with the reagent. The method is applicable within the limits of 0.01–0.1 mg. of iodide.

**Sample—Water.** If not too high in other salts dilute or concentrate as the case may be to 25 cc.

**Salt brines.** To a 50 cc. sample add 2 grams of ferric sulfate and render distinctly acid with sulfuric acid. To a receiver add 10 cc. of 1 per cent sodium hydroxide solution and 5 cc. of 3 per cent hydrogen peroxide. Steam distil the sample until no further evidence is shown of iodine distilling over. Evaporate the contents of the receiver to dryness and take up with 5 cc. of distilled water. Neutralize with 1:1 sulfuric acid, dilute to 25 cc. and use as sample.

<sup>40</sup> Cf. Roe E. Remington, J. F. McClendon and Harry von Kolnitz, *J. Am. Chem. Soc.* 53, 1245 (1931); R. G. Turner and Minna Z. Weeks, *Ibid.* 54, 829-30 (1932).

<sup>41</sup> N. A. Lange and L. A. Ward, *J. Am. Chem. Soc.* 47, 1000 (1925).

**Procedure**—Make the sample alkaline with 1 per cent sodium hydroxide solution. Add 10 cc. of 3 per cent hydrogen peroxide to oxidize nitrites. Evaporate to 20 cc., filter and wash with hot water. Neutralize with sulfuric acid, using litmus paper. Evaporate to slightly less than 30 cc. and divide into 2 equal parts.

To one add 0.5 cc. of a solution of 1 gram of *o*-tolidine in 150 cc. of 95 per cent alcohol, and dilute to 15 cc. Through the other pass hydrogen sulfide until saturated. Completely remove excess hydrogen sulfide by g, cool, and add 0.5 cc. of the *o*-tolidine solution. Dilute to 15 cc.

Make a series of standards containing 1–10 cc. of standard iodide solution. To each add 0.5 cc. of *o*-tolidine solution and dilute each to 15 cc. To the unknowns and standards add 5 cc. of 3 per cent hydrogen peroxide as quickly as possible and shake. Compare after 5 minutes. After 10 minutes the blue color changes to brown and precipitation of organic matter occurs. The unknown treated with hydrogen sulfide gives a total value for iodide and iodate. The other unknown gives a value for iodide only. By difference an estimation of the amount of iodate may be obtained.

**Standard**—Dissolve 0.0131 g. of potassium iodide in water and dilute to 1 liter. Each cc. contains 0.01 mg. of iodine.

#### IODIDE BY MERCUROUS CHLORIDE

The action of mercurous chloride may be used to form yellow to greenish black mercurous iodide from a sample iodide solution and the color produced by its adsorption on excess mercurous chloride then serves for its estimation.<sup>41</sup> Arsenic, gold, platinum, palladium, selenium and tellurium interfere. The method will estimate 0.003 mg. of iodine on 0.1 mg. of mercurous chloride. Because of the methods of volatilization or extraction iodine can be separated from all interfering elements.

**Procedure**—Transfer 5 cc. of distilled water to a beaker and add 0.1 gram of mercurous chloride. Mix well and add sufficient sample solution to produce a positive coloration on the mercurous chloride. Mix well and let settle. Compare the color of the precipitate with that of standards.

<sup>41a</sup> Gordon G. Pierson, *Ind. Eng. Chem., Anal. Ed.* 6, 437-9 (1934).

## IODIDE IN IODIZED SALT

This method is designed to show whether a particular sample maintains the standard of 0.0221 per cent as potassium iodide.<sup>42</sup>

**Procedure**—Dissolve 5 grams of sample in 25 cc. of water in a separatory funnel. In other funnels dilute 4.5, 5.0 and 5.5 cc. of standard to 25 cc.

To sample and standards add 5 cc. of syrupy phosphoric acid and 10 cc. of colorless carbon bisulfide. Add 20 cc. of 3 per cent hydrogen peroxide and extract for 5 minutes. Filter portions of the lower layers of solvents through cotton into similar comparison tubes and compare. More accurate results can be obtained by comparison in a micro colorimeter. Of a number of trade samples, all but 2 were reported to be deficient in iodine.

**Standard**—Dissolve 0.2214 gram of potassium iodide in water and dilute to 1 liter. This is equivalent to the standard which iodized salt should meet.

## IODINE BY THE STARCH-IODINE REACTION

The color developed by iodine and starch can be used for colorimetric estimation.<sup>43-45</sup> It has been reported as an unsatisfactory reaction for the purpose.<sup>46,47</sup> The basis of the color is probably adsorption rather than a true compound.<sup>48</sup> The adsorption may be of molecular iodine<sup>49</sup> or more probably of potassium triiodide<sup>50,51</sup> or hydriodic acid.<sup>52,53</sup> It readily

<sup>42</sup> William C. Geagley, *Am. J. Pub. Health* 19, 991-6 (1929).

<sup>43</sup> H. Q. Woodard, E. H. Quimby and H. R. Downes, *Am. J. Roentgenol. Radium Therapy*, 29, 308 (1933).

<sup>44</sup> H. Q. Woodard, *Ind. Eng. Chem., Anal. Ed.* 6, 331-3 (1934).

<sup>45</sup> R. G. Turner, *J. Am. Chem. Soc.* 52, 2768 (1930).

<sup>46</sup> Th. von Fellenberg, *Ergebnisse Physiol.* 25, 190 (1926).

<sup>47</sup> Roe E. Remington, J. F. McClendon and H. von Kolnitz, *J. Am. Chem. Soc.* 53, 1245 (1931).

<sup>48</sup> Cf. N. R. Dhar, *J. Phys. Chem.* 28, 125 (1924).

<sup>49</sup> S. W. Gorbatscheff and E. N. Winogradowa, *Z. phys. Chem.* 127, 63 (1927).

<sup>50</sup> E. Angelescu and J. Mirescu, *J. chim. phys.* 25, 327 (1928).

<sup>51</sup> J. B. Firth and F. S. Watson, *J. Soc. Chem. Ind.* 42, 308 (1923).

<sup>52</sup> M. J. Gramenitski, *Biochem. Z.* 185, 427 (1927).

<sup>53</sup> Staiger, *Z. Spiritusind.* 50, 300 (1927).

dissociates at low concentrations.<sup>54</sup> The color developed increases rapidly with increase of potassium iodide up to 0.35 *N*, then more slowly.

**Starch Reagent**—Make a paste of 2 grams of soluble starch and 30 cc. of cold water. Add this slowly to 70 cc. of boiling water and continue to boil for 5 minutes. Use only for 3–4 days before renewing.

**Procedure**—Transfer the sample to a 10 cc. or 25 cc. volumetric flask. To another flask add a known amount of standard. Dilute each to about 40 per cent of the volume of the flask. To each add 10 per cent potassium iodide solution to within about 1 cc. of the mark and mix. Add 1 cc. of starch solution and dilute to volume. Mix and compare.

**Standard**—Weigh out 0.25 gram of pure iodine and dissolve in 0.2 per cent potassium iodide solution. Dilute to 1 liter with 0.2 per cent potassium iodide solution. Each cc. contains 0.25 mg. of iodine. For greater accuracy standardize by thiosulfate titration.

<sup>54</sup> F. P. Treadwell, "Analytical Chemistry," 3rd ed., vol. II, p. 653. John Wiley and Sons, New York, N. Y. (1914).

## CHAPTER LVI

### FLUORIDES

#### FLUORIDES BY BLEACHING AN OXIDIZED TITANIUM SOLUTION

SMALL amounts of fluoride may be estimated by their action on a titanium solution which has been oxidized by the addition of hydrogen peroxide.<sup>1,2</sup> This method is more accurate than gravimetric methods for amounts below 2 per cent and will estimate amounts not detectable by gravimetric methods.

The procedure must be carefully standardized as there are numerous interfering factors. Changes of temperature produce important variations. The presence of alkali sulfates causes a bleaching action similar to that of fluoride. Heating or the presence of considerable free acid will restore part of the color bleached. The bleaching action increases with pH to an optimum at 1.5, then rapidly decreases to substantially 0 at pH 2.5.<sup>3</sup> Some organic matters interfere. This procedure has been applied to samples separated from interfering substances by volatilization.<sup>4,5</sup> Silica must be removed before the solution is made acid because of its reaction with fluoride. The acid solutions must not be allowed to stand in glass containers before development of color after they are acidified.

Phosphoric acid and aluminum also cause a bleaching action. Aluminum can be removed, but because of interference, determination of fluorides in phosphates is impractical by colorimetric methods unless the fluoride is distilled from the phosphates.<sup>6</sup> Iron causes an interfering color. Nitrates interfere.

Because of the complexity of the possible interfering factors a curve must be developed for the specific detail and method in use by the individual laboratory.<sup>7</sup> This must be standardized as to volume of

<sup>1</sup> G. Steiger, *J. Am. Chem. Soc.* 30, 219-25 (1908).

<sup>2</sup> H. E. Merwin, *Am. J. Sci.* [4] 28, 119-28 (1909).

<sup>3</sup> H. J. Wichmann and Dan Dahle, *J. Assoc. Official Agr. Chem.* 16, 612-9 (1933).

<sup>4</sup> E. Peyrot, *Ann. chim. applicata* 24, 74-8 (1934).

<sup>5</sup> See pp. 577-80.

<sup>6</sup> C. R. Wagner and W. H. Ross, *J. Ind. Eng. Chem.* 9, 1116-23 (1917).

<sup>7</sup> W. H. Adolph, *J. Am. Chem. Soc.* 37, 2500-15 (1915).

excess acid present, amount of sodium and potassium carbonates used and present in the final solution as sodium and potassium sulfates, and temperature of comparison, as well as in the volumes of the usual reagents added to develop the color. Properly standardized, the method will permit estimations down to 0.002 mg.

**Sample—Rock.**<sup>1</sup> Fuse a finely ground 2 gram sample with 10 grams of a mixture of equal parts of sodium and potassium carbonates. About 50 per cent of silica should be present. Dissolve the fusion in water after cooling. The solution should total about 100 cc.

Filter the aqueous solution from insoluble residue. Add 4 grams of ammonium carbonate and heat on a boiling water bath for 15 to 20 minutes to precipitate silica and aluminum. Cool, let stand 1 hour and filter. Evaporate the filtrate to about 25 cc. and filter again. Collect the filtrate in a 100 cc. volumetric flask. Nearly neutralize to phenolphthalein with 1:3 sulfuric acid and heat to drive off carbon dioxide. Cool and add more sulfuric acid to neutrality. Mix well.

This may be diluted to volume and an aliquot taken. In that case the comparison curve must have been prepared for a similar aliquot.

**Basic slag.**<sup>9</sup> Fuse 1 gram of basic slag at a dull red heat with 6 grams of a mixture of sodium and potassium carbonates in a covered platinum crucible. Heat for 1 hour, with frequent mixing. Avoid too high a temperature or manganese may be extracted and give the solution a yellow color. While still moderately hot drop the crucible with its contents into 200 cc. of water in a 400 cc. beaker. Digest on a sand bath for 2 to 3 hours. Remove the crucible when all the material is disintegrated and concentrate the liquid to 70–100 cc. Cool, add 3 or 4 grams of solid ammonium carbonate and let stand over night. Filter and wash the residue with a 5 per cent ammonium carbonate solution. If there is any question as to complete extraction of fluorides dry the residue and fuse again as a separate determination.

Evaporate the filtrate to dryness on a water bath. Take up with 50 cc. of water, filter, and evaporate to dryness. This removes silica, iron and aluminum. Add 50 cc. of water saturated with phenolphthalein and titrate hot with 4 *N* sulfuric acid. Do not let all the indicator color disappear or hydrofluoric acid may be lost. Boil after each addition of acid to expel carbon dioxide. When the end point is near put the solution into a 100 cc. flask and complete the titration with 0.1 *N* acid. Evaporate the solution to 50 cc. Add 0.6 gram of powdered silver

---

<sup>9</sup> R. G. Warren, C. T. Gimingham and H. J. Page, *J. Agr. Sci.* 15, 516 (1925).



sulfate to the hot solution with stirring. Set aside over night in a dark place and filter in the dark into a 100 cc. flask through double filter papers. Transfer the precipitate to the paper and wash carefully with 20 cc. of distilled water. Use the entire filtrate as a sample to maintain the salt concentration standard.

*Enamel.*<sup>9a</sup> Fuse the sample with a suitable amount of sodium carbonate. Extract the melt with water and filter. Add an excess of ammonium carbonate and boil to precipitate silica. Filter and concentrate to a suitable volume. Neutralize with 1:1 sulfuric acid to phenolphthalein using the indicator on a spot plate. Use this or an aliquot as sample. If phosphates or other interfering substances are present concentrate the fluoride by distillation.<sup>9b</sup>

**Procedure—Balancing Method.** To the sample or an aliquot add 10 cc. of 1:2 sulfuric acid and 3 cc. of 3 per cent hydrogen peroxide. No brown color should appear. Add 10 cc. of titanium sulfate solution containing 1 mg. of titanium dioxide per cc. Dilute to 100 cc. Read in a colorimeter against a solution containing the same amounts of sulfuric acid, hydrogen peroxide, and titanium sulfate added to 50 cc. of water, then diluted to 100 cc. As this standard contains no fluorides, its reading is taken as representing 100 per cent color development.

The amount of fluorides shown by the degree of fading must be read from a curve determined empirically with known amounts of fluorides. For preparation of a curve for minerals, fuse mixtures in which known weights of calcium fluoride are added to fluoride-free sample. Use the same procedure and either take the entire solution or a suitable aliquot according to what is to be done with the sample. For other samples mix the standard titanium solution with other substances known to be present and develop the color. Plot the values of color reading obtained with the standard titanium solution against the amount of fluoride present. This curve is only approximately a straight line.

**Standard Titanium Solution**—Treat 3 grams of pure recrystallized potassium titanium fluoride with concentrated sulfuric acid in a platinum crucible and evaporate nearly to dryness. Repeat several times and finally take to dryness on a sand bath. Extract the residue with 250 cc. of 1:4 sulfuric acid. When completely in solution dilute to 1 liter. Each cc. contains 1 mg. of titanium dioxide.

---

<sup>9a</sup> W. Dawidl, *Keram. Rundschau* 42, 607-8 (1934).

<sup>9b</sup> See pp. 577-80.

In case of question as to the accuracy of this standard solution determine the titanium gravimetrically by precipitation as titanium oxide with ammonium hydroxide.

#### FLUORIDES BY BLEACHING FERRIC THIOCYANATE

Ferric thiocyanate solution is changed from deep red to orange or yellow in the presence of fluorides. The color is inversely proportional to the amount of fluoride present and may be used for estimation of soluble fluorides in the absence of interfering substances.<sup>10</sup> The change in color is due to the removal of iron by combination with fluoride.<sup>11</sup> Sulfates and chlorides produce a small but similar effect. They must therefore be present only in small amounts or a correction made for them.

The alteration in color, illustrated in Figure 84, although definite and reproducible, is not a straight line function.<sup>12</sup> Caution must be exercised in its application to apply all necessary corrections, which often depend on the accuracy of other analyses.<sup>13</sup> Results by this method agree well with those by alizarin sodium sulfonate and by titration of distilled fluoride as hydrofluosilicic acid.<sup>13a</sup>

**Sample—Water.**<sup>12</sup> Neutralize the alkalinity of a 50 cc. sample in a comparison tube with 0.05 *N* nitric acid. If very alkaline use stronger acid so that the neutralized sample will not exceed 60 cc. Calculate the necessary amount of acid from the determination of alkalinity so that an indicator will not have to be added.

**Soil.**<sup>14a</sup> Fuse 5 grams of soil with 10 grams of a mixture of equal parts by weight of sodium carbonate and potassium carbonate. Dissolve the melt in water and evaporate to dryness on a water bath. Take up the melt with 75 per cent alcohol which dissolves all of the fluoride but relatively little of the carbonates and silicates. Filter and evaporate the filtrate to dryness. Take up with 95 per cent alcohol and filter. Evaporate to dryness and take up with water. Add 0.01 per cent silver sulfate solution carefully so that all chlorides, bromides and iodides are precipitated but no substantial excess is added. Filter and add an equal

V. K. Smitt, *Chem. Trade J.* 71, 325 (1922).

<sup>11</sup> Margaret D. Foster, *J. Am. Chem. Soc.* 54, 4464-5 (1932).

<sup>12</sup> Margaret D. Foster, *Ind. Eng. Chem., Anal. Ed.* 5, 234-6 (1933).

<sup>13</sup> Dan Dahle, *J. Assoc. Official Agr. Chem.* 17, 204-6 (1934).

<sup>13a</sup> H. V. Smith, *Ind. Eng. Chem., Anal. Ed.* 7, 23-5 (1935).

<sup>14a</sup> J. S. McHargue, *J. Assoc. Official Agr. Chem.* 18, 207-10 (1935).

volume of 95 per cent alcohol to precipitate sodium sulfate. Filter and carefully add 0.1 *N* barium hydroxide solution until the solution is just alkaline to phenolphthalein. Filter and evaporate to dryness. Take up the residue with 0.1 *N* hydrochloric acid and water. Filter if necessary, and use as sample, or dilute to a known volume and use an aliquot.

*Solutions containing Fluorides.* These may have been prepared in any of the usual ways, avoiding the presence of chlorides and sulfates

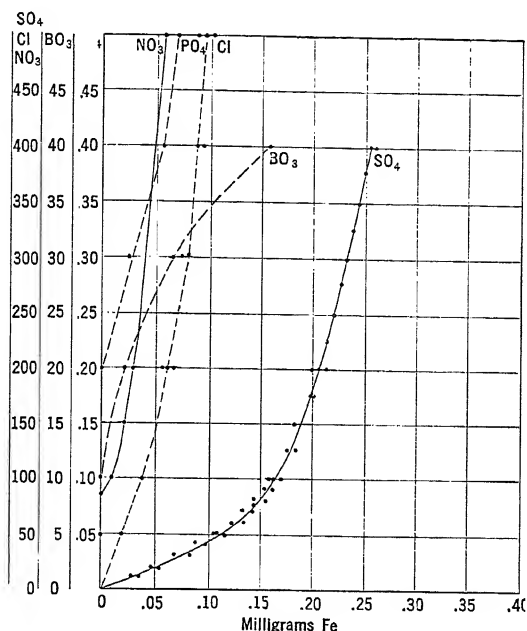


FIG. 83

Correction for NO<sub>3</sub>, SO<sub>4</sub>, Cl, BO<sub>3</sub> and PO<sub>4</sub> in Natural Waters for Fluoride Determination

as far as possible. For analysis dilute to a fluoride concentration between 0.0005 and 0.004 mg. per cc.

**Procedure**—To the sample add 5 cc. of a ferric chloride solution made up by titration to contain 0.075 mg. of iron per cc. and containing 30 cc. of *N* hydrochloric acid per liter. Add the necessary excess indicated by the curves, Figure 83, as necessary to be equivalent to the sulfate and chloride present in the sample. Add 10 cc. of a 0.24 per cent solution of ammonium thiocyanate, dilute to 75 cc. and mix.

Compare at once with a standard prepared from the same iron solution at the same time. Subtract the amount of iron found present from 0.375 mg., the amount added, and read from the curve, Figure 84, the amount of fluorides present. No correction need be made for sulfate or phosphate by addition of excess iron if less than 2.5 and 5.0 mg. respectively

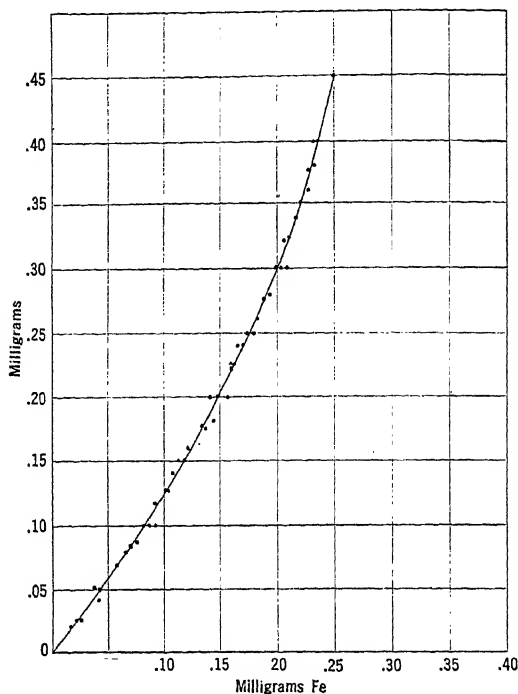


FIG. 84

Amounts of Fluorides in Mg. Equivalent to Iron Color Destroyed

are present. If more than 50 or 100 mg. are present, the method must be modified.

#### FLUORIDES BY BLEACHING FERRIC ACETYLACETONE

As in the preceding method for bleaching of ferric thiocyanate, the principle has been applied to the color of ferric salts with acetylacetone.<sup>15</sup>

<sup>15</sup> W. D. Armstrong, *Ind. Eng. Chem., Anal. Ed.* 5, 300-2 (1933).

As in that case, the measurement is by the power of the fluoride to combine with iron and thus prevent its development of the usual color. Similar experiments, not reported in detail, were conducted with the colors of ferric salicylate and the ferric compound of 8-hydroxyquinoline.

The fading caused by different amounts of fluoride is a straight line function. An equilibrium condition rather than binding of iron by all the fluoride apparently occurs. The slope of the line is altered by acidity and other factors. It is therefore necessary to use as standard a portion of sample to which a known amount of fluoride has been added, comparing this and the sample against the same colored solution. By so doing errors due to variations in acidity and due to reasonable amounts of impurities are minimized.

The presence of not more than 0.05 gram of sodium chloride and 0.1 gram of sodium sulfate is permissible in the aliquot used. Sodium nitrate up to 0.4 gram and silicic acid to saturation have no effect. Ions which form precipitates or undissociated compounds with ferric ion or fluoride must be absent. A very high degree of accuracy is attained.

If solids contain interfering substances, the fluoride must be volatilized with sulfuric acid in the presence of silica and absorbed<sup>15</sup> for accurate results.<sup>17-21</sup> This is the only type of method applicable in the presence of large amounts of phosphates.<sup>22</sup> Volatilization should be as the silicon fluoride.<sup>23</sup> In a procedure using perchloric acid,<sup>24</sup> apatite, rock phosphate, cryolite and feed ash were analyzed without prior treatment. Opal glass required preliminary fusion with sodium carbonate.

This method has been found more accurate because less susceptible to the effect of sulfate and chloride than the ferric thiocyanate method, measurements being with a photoelectric cell.<sup>25</sup>

**Sample—Solutions.** If the sample is alkaline, add a few drops of phenolphthalein solution to a known volume and add 0.1 *N* hydrochloric

<sup>17</sup> Offerman, *Z. angew. Chem.* 3, 615 (1890).

<sup>18</sup> F. P. Treadwell and A. A. Koch, *Z. anal. Chem.* 43, 469-506 (1904).

<sup>19</sup> Cary R. Wagner and William H. Ross, *J. Ind. Eng. Chem.* 9, 1116-23 (1917).

<sup>20</sup> G. A. Shuey, *J. Assoc. Official Agr. Chem.* 11, 147-9 (1928).

<sup>21</sup> George R. Sharpless and E. V. McCollum, *J. Nutrition* 6, 163-78 (1933).

<sup>22</sup> D. S. Reynold, W. H. Ross and K. D. Jacobs, *J. Assoc. Official Agr. Chem.* 11, 225-36 (1928).

<sup>23</sup> L. Szegő and B. Cassoni, *Giorn. chim. ind. applicata* 15, 599-602 (1933).

<sup>24</sup> H. H. Willard and O. B. Winter, *Ind. Eng. Chem., Anal. Ed.* 5, 7-10 (1934).

<sup>25</sup> L. V. Wilcox, *Ind. Eng. Chem., Anal. Ed.* 6, 167-9 (1934).

or nitric acid until acid. Boil and continue to add acid until the pink color no longer returns on further boiling. Add 0.1 *N* sodium hydroxide solution until just alkaline and let cool in a stoppered tube. When cool, again render just acid and add 1 drop of 1:100 acid. Dilute to a known volume.

Figure 85 illustrates the evolution apparatus. Joints B and C are of ground glass, D is an adapter. Figure 86 shows the complete ap-

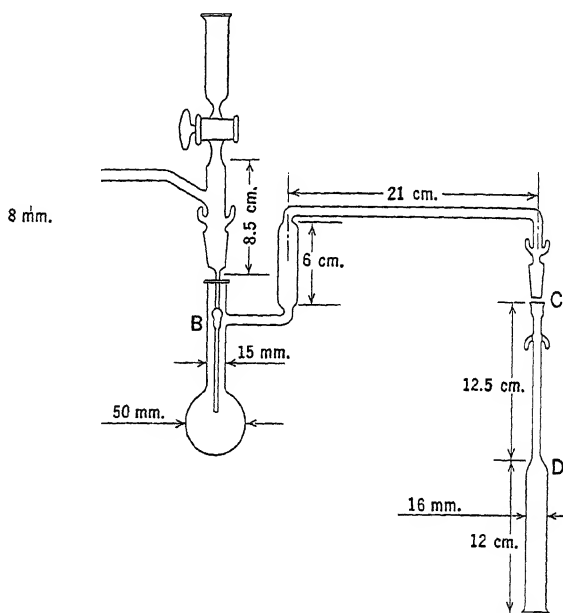


FIG. 85

Details of Evolution Apparatus for Fluorine

paratus. Tube B is of rubber and carries compressed air. The Milligan wash bottle D contains concentrated sulfuric acid. Four tubes E are 25 x 100 mm. and are filled with calcium chloride. Tube F is of similar size and contains phosphorus pentoxide and glass beads. Glass wool fills G. Tubes H and M are of rubber, controlled by clamps I and N. The receiver L is made from a 25 x 200 mm. test tube. Pressure is regulated by O. Trap P is connected to a water pump. The apparatus is so suspended that K can be shaken.

For use, oven-dry the evolution apparatus shown in Figure 85. Mix

the sample with 2 grams of powdered glass <sup>26</sup> in the bowl of the apparatus. If more than 3 grams of sample are used, increase the glass to 3 grams. Assemble the apparatus, except the adapter and receiver. Connect the air line at B and blow air through the apparatus for 30 minutes. A steady stream of bubbles should form in the wash bottle. Heat the evolution flask occasionally, starting with the end attached to H to remove moisture absorbed on the walls. At the end of that period, place 20 cc. of water in the receiver and connect it by the adapter D, Figure 85. Change the apparatus from pressure to suction, regulate the flow at C until 1 bubble per second forms in the receiver, and continue this rate

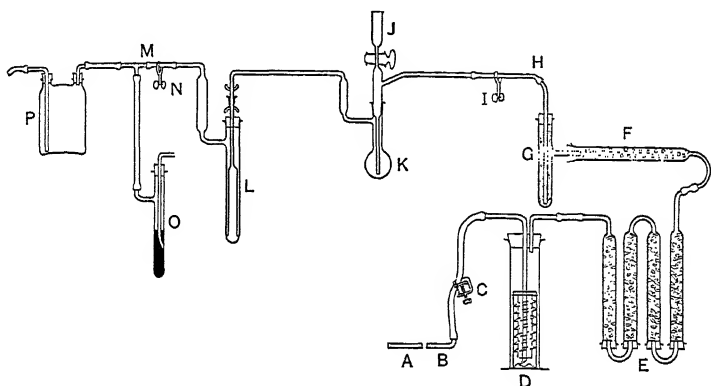


FIG. 86

Evolution Apparatus for Fluorine

of flow until the run is completed. Heat the apparatus with a free flame again, including the joint of the adapter. Add 20 cc. of concentrated sulfuric acid without excess sulfur trioxide <sup>7</sup> to the sample, leaving some in J as a seal. Mix this with the sample and heat by a paraffin bath. The temperature should be 140–150° for one and a half hours and 175° for an additional 2 hours. Shake the contents of the flask at 10 minute intervals to mix. If a white sublimate forms on the delivery tube, heat it with a free flame until it is carried over into the adapter. This should not occur unless the sample is heated too rapidly.

At the end of the operation close N and I, open the stopcock at J and disconnect the apparatus. Rinse the adapter, including the silicic acid on its lower end, and the contents of the receiver into a flask. Dilute the solution to about 200 cc. and heat to boiling for 5 minutes.

<sup>26</sup> J. Casares, *Anales. soc. españ. fs. quim. (technica)* 27, 290-301 (1929).

Add a few drops of phenolphthalein solution and add 0.1 *N* sodium hydroxide solution until the color of the indicator persists on further boiling. Cool the solution and dilute to a known volume. Use this, or an aliquot, as sample.

In work with 0.04–0.06 gram of fluoride, recovery was correct within 0.33 per cent.

**Procedure**—Add to each of three 250 cc. volumetric flasks 1 cc. of an iron solution containing 0.3 mg. of iron. This solution must be protected from light and not more than 2 to 3 hours old. Add 1 cc. of a 0.5% per cent, freshly distilled solution of acetylacetone to each.

To one flask add an aliquot of the sample containing not more than 0.25 mg. of fluoride. To a second flask add the same size aliquot of sample and 1 cc. of a solution containing 0.1 mg. of fluoride as sodium fluoride per cc. Make no addition to the third, or standard, flask. Dilute each to volume.

Set the standard in the left hand cup at a depth of 20 mm. and compare the sample solution against it by balancing. Similarly compare the sample to which 0.1 mg. of fluoride was added. Each value should be the average of 20 readings.

Calculate the results according to the following equation.

$$F = \frac{0.1}{Y - X}$$

F = Fluoride content of the entire sample solution in grams.

X = Reading of sample solution.

Y = Reading of sample solution to which 0.1 mg. of fluoride was added.

D = Ratio of total sample to the aliquot used.

**Standard**—Dissolve 0.221 gram of sodium fluoride in water and dilute to 1 liter. Each cc. contains 0.1 mg. of fluoride in the form of sodium fluoride. For 0.02 mg. per cc. or 20 p.p.m., dilute 100 cc. of the above solution to 500 cc., or for 0.01 mg. per cc. or 10 p.p.m. dilute 100 cc. of the above solution to 1 liter.

#### FLUORIDES BY REACTION WITH ALIZARIN SODIUM SULFONATE AND ZIRCONIUM NITRATE

The reaction of fluoride ion with alizarin sodium sulfonate and zirconium nitrate in acid solution to produce a series of colors ranging from



pink to yellow-green can be used for estimation of fluoride. The original complicated method for sea water,<sup>28</sup> which allows for excess chlorides, has been much broadened in its applicability.<sup>29</sup>

Hydrochloric acid alone is not suitable for acidification of the sample, but the use of both hydrochloric and sulfuric acids for this purpose eliminates the effect of sulfate within reasonable limits. Results indicate that 500 p.p.m. of chlorides, sulfates, bicarbonates, sodium, calcium and magnesium, 200 p.p.m. of manganese, 50 p.p.m. of silicates, 5 p.p.m. of phosphates, boron, copper and iron, and 2 p.p.m. of sulfides do not interfere.

The color after cooling for 4 hours is permanent for a day. Sample and standard must be treated at the same time. Excess organic matter and phosphates tend to throw some indicator out of solution. It can be redispersed when these materials are present only in small amounts, but in extreme cases they must be removed. Results are accurate to 0.2 p.p.m. of fluoride.

**Reagent**—Dissolve 0.17 gram of alizarin sodium sulfonate in 100 cc. of water. Dissolve 0.87 gram of crystallized zirconium nitrate in 100 cc. of water. Mix the 2 solutions with constant stirring. Shake at intervals and let stand over night. Dilute 20 cc. of this stock solution to 100 cc. for use. When not in use, store concentrated and dilute reagent in a cool, dark place.

**Procedure**—*Water*.<sup>29,30</sup> Transfer a 100 cc. sample to a 250 cc. Erlenmeyer flask. In similar flasks place 0, 2.5, 5.0, 7.5, 10, 15, 20, 25 and 30 cc. of standard fluoride solution containing 0.01 mg. of fluoride per cc.<sup>31</sup> Dilute each standard to 100 cc. with distilled water. Each cc. of standard when so diluted is equivalent to 0.1 p.p.m.

To each standard and sample add 2.0 cc. of 3 *N* hydrochloric acid, 2.0 cc. of 3 *N* sulfuric acid and 2.0 cc. of diluted reagent. Bring the contents of the flasks rapidly to boiling on a hot plate. Remove soon after boiling starts. They should not boil vigorously or simmer for a long time. After cooling for at least 4 hours transfer the solutions to 100

<sup>28</sup> Thomas G. Thompson and Howard Jean Taylor, *Ind. Eng. Chem., Anal. Ed.* 5, 87-9 (1933).

<sup>29</sup> J. M. Sanchis, *Ind. Eng. Chem., Anal. Ed.* 6, 134-5 (1934).

<sup>30</sup> Cf. Guy Barr and A. L. Thorogood, *Analyst* 59, 378-80 (1934).

<sup>31</sup> See p. 580.

cc. Nessler tubes and dilute to volume. Mix and compare the unknowns with the standards.

If a moderate amount of reddish precipitate appears in the flasks after cooling, disperse it by swirling the flask prior to transfer to the Nessler tubes.

*Sea Water.*<sup>33</sup> As standards use 8, 10, 11, 12, 13, 14, 15 and 16 cc. of a standard fluoride solution containing 0.01 mg. of fluoride per cc.<sup>33</sup> When diluted to 100 cc. these represent 0.8–1.6 mg. of fluoride per liter. Dilute these with distilled water and a mixed salt solution to give a chlorinity equal to that of the sample. Use a solution of 37.9 grams of sodium chloride and 8.25 grams of magnesium sulfate per liter for this adjustment. This has a chlorinity of 23 grams of chlorine per liter and a ratio of sulfate to chloride analogous to that of sea water.<sup>34</sup>

Transfer 100 cc. of sample and standards as above to 250 cc. Erlenmeyer flasks. To each add 2 cc. of 6 *N* hydrochloric acid. If the chlorinity is 14 to 17 per mille, add 1.5 cc. of indicator solution. For each unit-increase above 17 per mille add 0.075 cc. of indicator and for each unit-decrease below 14 per mille decrease the indicator by 0.038 cc.

After adding the indicator, bring the solutions to the boiling point to facilitate reaction. When cool, transfer to 100 cc. Nessler tubes, and adjust to volume. Let stand 4 hours and compare. If the chlorinity of the sample is less than the standard, correct the results by adding 0.054 mg. of fluoride for each unit. Subtract if that of the sample is higher.

#### FLUORIDES BY ZIRCONIUM OXYCHLORIDE AND 1,2,4-TRIHYDROXYANTHRAQUINONE

By use of the duplication method, small amounts of fluorides can be estimated by the orange color produced with zirconium and purpurin, 1,2,4-trihydroxyanthraquinone.<sup>35</sup> The technique is such as to make it a border line case between colorimetric and volumetric methods. The method is accurate to 0.002 mg. of fluoride in a sample containing up to 0.05 mg. By modification of the volumes of reagent and standard its range could be extended.

**Sample**—Acidify a known volume with concentrated hydrochloric

<sup>33</sup> See p. 580.

<sup>34</sup> T. G. Thompson, *J. Am. Chem. Soc.* 50, 681-5 (1928).

<sup>35</sup> I. M. Kolthoff and Maurice E. Stansby, *Ind. Eng. Chem., Anal. Ed.* 6, 118-21 (1934).

acid until it is 2 *N* and dilute to a known volume with 2 *N* hydrochloric acid. At that volume it should contain not more than 0.05 mg. of fluoride in a 2 cc. sample.

**Reagent**—Mix slowly with shaking 9 mg. of 1,2,4-trihydroxyanthraquinone in 30 cc. of 95 per cent ethyl alcohol and 0.16 gram of zirconium oxychloride in 6 *N* hydrochloric acid. Add 620 cc. of concentrated hydrochloric acid to the mixture and dilute to 1 liter with water.

**Procedure**—Transfer 10 cc. of the reagent to each of 2 tubes. Add 2 cc. of the sample to one. To the standard tube add 2 cc. of 6 *N* hydrochloric acid. Add 2.4 cc. of a fluoride solution containing 0.02 mg. of fluoride ion per cc.<sup>35a</sup> to the standard tube. Mix and add the fluoride solution containing 0.02 mg. of fluoride per cc., 8 *N* hydrochloric acid and water to the sample until the color, volume and acidity of the standard are matched. Subtract the volume of standard fluoride solution added to the sample from the volume used in the standard tube compared to give the volume of standard equivalent to the sample.

#### FLUORIDES BY ZIRCONIUM NITRATE AND 1,2,5,8-TETRAHYDROXYANTHRAQUINONE

The color of fluoride with zirconium and 1,2,5,8-tetrahydroxyanthraquinone, quinalizarine, can be used for its estimation.<sup>36</sup> Interfering ions do not occur in sufficient quantities in natural waters to alter the result by more than 10 per cent. Less than 20 p.p.m. of sulfates have no effect and are easily removed with barium chloride. Barium ion has no effect. Iron above 10 p.p.m. alters the color. Aluminum up to 0.2 p.p.m. has no effect. As much as 0.6 p.p.m. alters the result by 0.1 p.p.m. of fluoride. More than 0.5 p.p.m. of aluminum is rarely present in natural waters. Phosphates greater than 0.4 p.p.m. alter the color with an easily recognizable change. If excessive amounts of foreign ions are present, the fluoride is distilled.<sup>37</sup>

In over 200 natural waters, results by distillation and by direct determination agreed on an average within 10 per cent. The initial color fades rapidly during the first 15 minutes, but only slowly thereafter. This property dictates extreme caution in standardizing the conditions of treating the samples and standards.

<sup>35a</sup> See p. 580.

<sup>36</sup> O. M. Smith and Harris A. Dutcher, *Ind. Eng. Chem., Anal. Ed.* 6, 61-2 (1934).

<sup>37</sup> See pp. 577-80.

**Reagent**—Dissolve 0.14 gram of 1,2,5,8-tetrahydroxyanthraquinone in 0.3 per cent sodium hydroxide solution and dilute to 100 cc. with the same alkaline solution. Dissolve 0.87 gram of zirconium nitrate in water and dilute to 100 cc. As reagent add 1.5 cc. of each to 100 cc. of water and mix.

**Sample**—*Water*. To 100 cc. of sample add 5 cc. of a 2 per cent barium chloride solution. Let settle for several hours and filter if necessary.

**Procedure**—To 50 cc. of sample freed from sulfates add 3 cc. of 1:1 hydrochloric acid and 5 cc. of reagent. Mix and compare after 20 minutes with a series of standards similarly prepared at the same time. As standards use up to 10 cc. in 1 cc. steps of a solution containing 10 p.p.m. of fluoride,<sup>38</sup> diluted in each case to 50 cc. with distilled water.

#### FLUORIDES BY ESTIMATION OF LEAD SULFIDE

Fluorides may be determined according to a rather lengthy and complicated procedure by their ratio to colloidal lead sulfide obtained from lead fluoride.<sup>39</sup> The color of the lead sulfide solution is compared with that of a standard solution of lead sulfide.

After obtaining the sample in solution by suitable treatment, the compounds of fluorine are precipitated together with barium sulfate. In a specially designed, hermetically sealed apparatus the fluorine compounds are decomposed and then volatilized as hydrofluoric acid and fluosilicic acid. The acids are condensed, and absorbed by solid potassium hydroxide. They are again dissolved and reprecipitated with barium sulfate. Hydrofluoric acid is evolved and condensed on crystals of lead glass, forming lead fluoride. The latter is dissolved in a saturated solution of potassium chlorate, the lead precipitated as colloidal lead sulfide and determined colorimetrically.

The ratio of lead to fluoride has to be determined empirically with known amounts of fluorides. For amounts of fluorine below 1 mg. the ratio is highly variable, becoming as low as F:1.14Pb with 0.05 mg. of fluorine due to retention in the distillation process. For amounts between 1 and 2 mg. the ratio varied only from F:2.51Pb to F:2.57 Pb. This is for a particular lot of lead glass and any particular lot must be standardized.

**Sample—Water.** Amounts of 0.25–1.0 mg. of fluoride per liter must be separated to obtain them in more concentrated form. Take 1 liter as sample. Add hydrochloric acid or potassium hydroxide solution until only faintly alkaline. Add 0.3–0.4 gram of crystallized sodium sulfate and when completely in solution a slight excess of 10 per cent barium chloride solution. Not over 5 cc. should be required. Evaporate to dryness at 100°. Add sufficient cold water to redissolve the soluble salts and add an equal volume of 95 per cent alcohol. Transfer to a centrifuge tube and wash with 65 per cent alcohol until the washings no longer give a test for chloride. The residue contains the fluoride together with sulfate, silicate, phosphate, borate and other salts of barium.

**Water. Alternative Method.** If the amount of sulfate present is too great for treatment by the previous method add to 1 liter of sample 5 cc. of 20 per cent magnesium chloride solution. Add 1 gram of ammonium phosphate dissolved in 10 cc. of distilled water and concentrated ammonium hydroxide to distinct alkalinity. Evaporate nearly to dryness, take up with 10 cc. of water and evaporate to dryness.

**Carbonates.** If soluble in concentrated sulfuric acid grind to a fine powder, and weigh 2 grams directly as sample.

**Silicates.** Fuse 2 grams with sodium and potassium carbonates according to the procedure under fluorides by their action on oxidized titanium solutions.<sup>40</sup> After removal of silica, iron and aluminum evaporate to dryness.

**Vegetable or Animal Matter.** Mix well with 10 per cent of slaked lime, using the minimum amount of water. Dry at 110° and grind to a fine powder. Calcine at 550–600° to remove organic matter. The ash should be definitely alkaline. Take up with water and carefully add 1:3 hydrochloric acid until effervescence ceases. Add 10 per cent potassium hydroxide solution until definitely alkaline and proceed as for the first method of water analysis, adding sodium sulfate next without filtration.

**Procedure—**A special type of equipment is required to concentrate the fluoride further, as shown in the accompanying Figure 87.

Place the dry sample in the bottom of the gold crucible of 50–55 cc. capacity. Place 1–1.5 cc. of concentrated sulfuric acid in the dish, C, above it, supported by S. In the upper platinum or gold dish, P, put 0.6 gram of pure potassium hydroxide moistened with water. Close so as to seal hermetically. Shake gently to upset the dish of sulfuric acid. The

<sup>40</sup> See p. 572.

hydrofluoric acid or  
potassium hydroxide.

During th

cool the

de evolved is absorbed by the  
piete reaction heat for 2 hours at  
stream of water through the con-  
D, and condense any vapors. The

chloride have now been

present fluoborate remains in the precipitate  
residue in water and precipitate the sulfate with barium chloride. Repeat  
the treatment as sample to recover the fluoride.

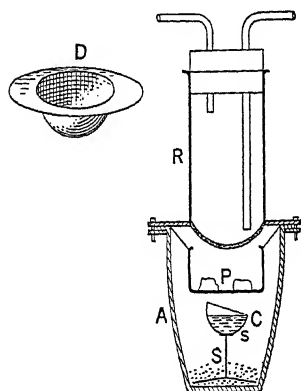


Fig. 87

Apparatus for Separation of  
Traces of Fluorides

chloride or

d w

Dissolve the potassium fluoride from  
the dish in 10 cc. of water and heat to  
boiling for 1 minute to decompose fluo-  
silicate. Add *N* hydrochloric acid until  
nearly neutralized. Add 1 drop of 1 per  
cent phenolphthalein solution and 3 to 4  
grams of ammonium carbonate. Evap-  
orate to dryness. Take up with 10 cc.  
of water and filter out silica. To the fil-  
trate add 1-2 cc. of *N* sodium sulfate  
solution for every 2-3 mg. of fluoride  
expected. Add 10 per cent barium ni-  
trate solution in slight excess and evap-  
orate to dryness. Take up with 10 cc.  
of water and add 10 cc. of 95 per cent  
alcohol. Transfer to a centrifuge tube  
and wash with 65 per cent alcohol so long  
as detected in the washings. Dry the  
quantitative estimation.

at previously described. The  
amount of sample to be used in the bottom. Th  
0.25 cc. of sulfuric acid. In place of the potass  
2.5-3 grams of coarsely ground lead-glass crystals wet with  
of water. Close, spill the dish of sulfuric acid and heat at 140° for 5  
hours, keeping the cover cooled. Fluoride unites with the lead to form  
no sulfuric acid is volatilized. A trace  
removed,  
ide. To  
with bc

per cent alcohol. This removes a trace of lead chloride or nitrate without dissolving the fluoride. If there is visible evidence of solution of the coating over the crystal by the alcohol, discard the determination, as too much chloride and nitrate are present.

To dissolve the lead fluoride without attacking the lead glass, mix 1 part of a saturated solution of potassium chlorate with 4 parts of water. Heat the crystals with 10 cc. of this solution on a water bath, filter and wash with 10 cc. more of the same solution. Dilute the filtrate to 25 cc. with the same solution.

To this add 2 to 3 drops of a 1 per cent gelatine solution and pass a few bubbles of hydrogen sulfide through. Compare with a standard and convert the reading in terms of lead sulfide to fluoride. To obtain the latter, results with known weights of fluoride against the lead glass used must be available.

**Standard Lead Solution**—Prepare a standard lead solution by dissolving 0.345 gram of crystallized lead nitrate in a solution of 1 part of a saturated solution of potassium chlorate and 4 parts of water. Dilute to 100 cc. with the same solution. Each cc. contains lead equivalent to 1 mg. of fluorine. In use however it must be standardized with a known fluoride against the lead glass used since in practice instead of a ratio of 1 : 2.158, that found was of the order of 1 : 2.51.

## CHAPTER LVII

### CHLORATES AND PERCHLORATES

#### CHLORATES BY ANILINE HYDROCHLORIDE

OXIDATION of aniline hydrochloride is a general reaction for estimation of small amounts of many oxidizing agents. A violet color develops at once, but changes to blue. The method is applicable to chlorates in the absence of other oxidizing agents.<sup>1</sup> The same color is produced by any other oxidizing agent capable of oxidizing aniline hydrochloride under the conditions of the method. The method will detect 0.007 mg. of potassium chlorate.

These interfering oxidizing agents include chlorine, hypochlorites, chlorates, hypobromites, bromates, iodates, hydrogen peroxide, peroxides of sodium, barium, manganese and lead, chromates and bichromates, manganates and permanganates, vanadates, ferricyanides and per salts in general. Substances more easily oxidized than aniline interfere. These include unstable chlorides, nitrites, citrates, tartrates, saccharates, a large number of organic reducing compounds, ferrous compounds, arsenites, sulfites, etc.

Preparation of the reagents to within 2 cc. of the final volume, treatment with 2 drops of concentrated potassium chlorate solution and finally diluting to volume has been recommended.<sup>2</sup> The resulting precipitate is filtered after 18 hours to give a reagent which is more dependable.

**Reagents**—Two concentrations of reagents are used according to the strength of the solution being examined.

*Solution A.* Dissolve 5 grams of pure aniline hydrochloride in 100 cc. of 1:2 hydrochloric acid. This is suitable for quantities of chlorate between 0.1 and 2 mg. in 5 cc.

*Solution B.* Dissolve 5 grams of pure aniline hydrochloride in 100 cc. of 1:3 hydrochloric acid. This is suitable for quantities of chlorate between 0.5 and 7 mg. in 5 cc.

---

<sup>1</sup> J. F. Virgili, *Ann. chim. anal. chim. appl.* **14**, 85-91 (1909).

<sup>2</sup> R. A. Jones, *Analyst* **56**, 807 (1931).



**Procedure**—Neutralize 5 cc. of the sample solution with acetic acid or with sodium carbonate. Place in a comparison tube with a suitable amount of 0.5 per cent potassium chlorate in another tube, diluted to 5 cc.

To sample and standard add 20 cc. of Solution A or B, according to the strength anticipated. Compare after 25 minutes if A is used, or after 15 minutes if B is used. They may be compared by dilution with an accuracy stated to be 1 per cent.

#### CHLORATES BY AMMONIUM THIOCYANATE

The reaction of chlorate ion to oxidize ammonium thiocyanate to a lemon to cadmium yellow <sup>2a</sup> may be utilized on test papers for estimation of chlorate.<sup>2b</sup> Only a drop of sample is needed and it need not be clarified. As would be expected the method is only roughly quantitative. It will detect 0.01 mg. of chlorate per cc. The color is due to canarine and pseudothiocyanic acid with small amounts of hydropseudothiocyanic acid and isoperthiocyanic acid. A similar color is produced by bromates, iodates, peroxides, persulfates, hypohalites and halogens. Perborates and borax do not interfere. Permanganates and dichromates mask the color and iron, cobalt, copper and molybdenum produce characteristic colors. The solution may be 0.1 *N* with sulfuric, nitric, acetic or oxalic acids, or with sodium hydroxide solution. It will detect 0.1 mg. of sodium chlorate in 100 mg. of sodium chloride. Large amounts of nitrate, phosphate, sulfate, cyanide, thiosulfate and sulfite limit the sensitivity to about 0.1 mg. per cc.

**Thiocyanate Papers.**—Impregnate Whatman No. 1 filter paper with 3 *N* ammonium thiocyanate solution. Dry with maximum air circulation out of contact with metal, at not over 70°. Protect from light and dust. A slight pink color due to a trace of iron will disappear on heating for use.

**Procedure**—Dry the paper at 60° for 10 minutes and cut the part showing no discoloration into strips. To these strips add a drop of the sample solution and of suitable standards. Heat on a clean glass surface at 95–105° until no further intensification of color occurs. This is usually 5–30 minutes. Compare the sample with the standards for estimation of the chlorate content.

<sup>2a</sup> P. Poch, *Anales soc. españ. fís quím.* 20, 662 (1922).

<sup>2b</sup> H. R. Offord, *Ind. Eng. Chem., Anal. Ed.* 7, 93-5 (1935).

## PERCHLORATES BY METHYLENE BLUE

Methylene blue gives a violet precipitate with perchlorates which is soluble in hot water.<sup>3,3a</sup> After treatment with a standard excess of methylene blue solution, the paler the color the greater the amount of perchlorate originally present. This inverse relation is used for colorimetric estimation by comparison with a series of standards. Persulfates give a rose precipitate. By allowing to stand for the precipitate to settle the color of the supernatant liquid can be compared,<sup>4</sup> or by addition of a large excess of zinc sulfate solution precipitation can be prevented and the comparison made after 15 minutes.<sup>5</sup> The reaction is applicable to other dyes containing the quaternary ammonium group such as crystal violet and malachite green.<sup>6</sup>

Iodides interfere but can be removed. Fluorides, chlorides, hypochlorites, chlorates, bromides, bromates, iodates and periodates, sulfates, nitrites, nitrates, phosphates, borates, perborates, carbonates and percarbonates do not interfere. Acid chromates interfere but may be precipitated by lead acetate in neutral solution.<sup>7</sup> A precipitate is also given by dilute acid solutions, permanganates, ferricyanides, metavanadates, molybdates and tungstates.

More than 5 per cent of sodium nitrate or 10 per cent of potassium nitrate interferes by precipitating some of the methylene blue. The method is intended to be used for relatively large amounts of perchlorate, such as are found in Chili saltpeter, 0.1 to 7 per cent. Chlorates containing perchlorate require a special procedure.

**Sample—Solid Salts.** Dissolve 20 grams of sample salt in water. If iodides are present add moist silver oxide to remove them. Filter and dilute to 100 cc.

**Plating baths.** Precipitate chromates from a definite volume by addition of excess lead acetate. A slight excess of the latter does no harm. Dilute to a known volume.

**Procedure without Zinc Sulfate<sup>4</sup>**—For less than 0.2 per cent perchlorate take 10 cc. of the prepared sample solution, for 0.2 to 0.5 per

<sup>3</sup> K. A. Hofmann, R. Roth, K. Hobald and A. Metzler, *Ber.* 43, 2624-30 (1910).

<sup>3a</sup> John H. Yoe, Private Communication, reports unsatisfactory results by this method.

<sup>4</sup> A. Monnier, *Arch. sci. phys. nat.* 42, 210-6 (1916).

<sup>5</sup> F. L. Kahn, *Z. angew. Chem.* 39, 451-4 (1926).

<sup>6</sup> Junck and Kupper, *Caliche* 8, 159-68 (1926).

<sup>7</sup> O. S. Fedorova, *J. Russ. Phys. Chem. Soc. Chem. Part* 59, 265-82, 509-20 (1927).

cent take 5 cc. and for over 0.5 per cent take 1 cc. Transfer this to a test tube and dilute to 20 cc.

Put portions of 1–5 cc. of a 0.1 per cent solution of potassium perchlorate into the same sized test tubes as used for the sample. To these standards add the same volume of 20 per cent perchlorate-free solution of the same kind of salt as is being examined. For examination of Chili saltpeter this would be pure sodium nitrate. Dilute each to 20 cc.

To sample and standards add 1 cc. of a 0.3 per cent aqueous solution of methylene blue. Let sample and standards stand for several hours in a cool place for precipitation. Compare the color of the sample with the series of standards prepared at the same time.

**Procedure with Zinc Sulfate**—*Perchlorate over 0.3 per cent.* Mix 5 cc. of a 0.032 per cent solution of methylene blue with 20 cc. of a 50 per cent solution of crystallized zinc sulfate. Add 0.2 cc. of the prepared sample solution, mix, and compare after 15 minutes with standards similarly treated. The colors obtained will correspond to the following series:

0%	Dark blue
0.1%	Slightly lighter
0.1–0.3%	Decreasing color intensity and increasing turbidity
0.4–0.7%	Reddish violet with gradually decreasing turbidity
0.7–1.0%	Further small changes.

The sensitivity is improved by comparison through a yellow glass filter. These standards keep well if properly protected and shaken before use.

*Perchlorate 0.05 to 0.3 per cent.* Mix 0.1 cc. of 1.6 per cent methylene blue solution with 25 cc. of 50 per cent crystallized zinc sulfate solution. Add 0.2 cc. of the prepared sample solution and compare after 15 minutes with a series of standards similarly prepared.

*Perchlorate under 0.05 per cent.* Mix 0.1 cc. of 1.6 per cent methylene blue solution with 25 cc. of saturated zinc sulfate solution. Add 0.2 cc. of the prepared sample solution. Compare with a series of standards similarly prepared. Amounts of the order of 0.02 to 0.04 per cent are discernible by this procedure.

*Chlorates containing Perchlorate.*<sup>7</sup> Prepare a reagent by mixing 0.1 cc. of 1.6 per cent methylene blue solution with 25 cc. of 50 per cent crystallized zinc sulfate solution. To 0.2 cc. of prepared sample solution,

or 0.1 cc. of sample solution and 0.1 cc. of water, add 0.1 cc. of 40 per cent potassium nitrate solution. Add 5 cc. of reagent and mix. Compare with standards.

By using a more concentrated zinc sulfate solution as small an amount as 0.1 per cent of perchlorate in potassium chlorate can be detected.

**Standard**—Dissolve 0.1393 gram of potassium perchlorate in water and dilute to 1 liter. Dilute 100 cc. of this solution to 1 liter. Each cc. of the first solution contains 0.1 mg. of chlorate radical and each cc. of the final solution 0.01 mg.

#### PERCHLORATES BY NITROSODIMETHYLANILINE

The action of perchlorates on nitrosodimethylaniline is adapted to estimation of perchlorates, particularly in Chile saltpeter.<sup>4</sup> Iodides must be removed with silver oxide. Iodates and periodates do not interfere.

**Procedure**—Dissolve a 1 gram sample in 20 cc. of water. If iodides are present add sufficient freshly precipitated silver oxide to remove them and filter. Transfer to a Nessler tube. Add 2 cc. of a 0.1 per cent solution of nitrosodimethylaniline in water, mix and let stand. Prepare a series of standard tubes at the same time using 1–10 cc. of the standard perchlorate. Treat in the same way and compare after allowing several hours for full development of color.

## CHAPTER LVIII

### SULFIDES

#### SULFIDES BY *p*-AMINODIMETHYLANILINE

SULFUR present as sulfides which can be decomposed with acid, when distilled into a solution containing hydrochloric acid and an oxidizing agent, reacts with *p*-aminodimethylaniline to give methylene blue.<sup>1</sup> The method is applicable to the analysis of iron,<sup>1,2</sup> food, tissue, sewage and mineral waters.<sup>3</sup> Interference by organic matter, alkalinity, nitrites and nitrates is avoided.<sup>4</sup>

Methyl and ethyl mercaptans are not absorbed by the zinc acetate solutions and do not interfere. The absorption of hydrogen sulfide by zinc acetate solution is quantitative under the conditions specified. Further distillation within reasonable limits does not alter the results. No measurable amount of hydrogen sulfide comes over after the first 15 minutes. The temperature and the concentration of hydrochloric acid must be kept constant. As little as 0.01 mg. of sulfide sulfur can be detected.

**Evolution Cylinder**<sup>3</sup>—Fit a tall cylinder from which the hydrogen sulfide is to be evolved with a 3-hole rubber stopper. Fit a delivery tube connected to a carbon dioxide cylinder into 1 hole. This is also connected with a manometer filled with a zinc chloride solution of sp. g. 2.0. The pressure registered is half that if water were used. Into a second hole put a dropping funnel containing 50 cc. of 1:1 hydrochloric acid. Into the third hole put an exit tube which also acts as a delivery tube for the hydrogen sulfide. This delivery tube extends beneath the surface of 30 cc. of a 0.6 per cent zinc acetate solution contained in a 100 cc. distilling flask used as the absorption vessel. Connect the side arm of this flask to a delivery tube extending beneath the surface of 20 cc. of a 0.6 per cent zinc acetate solution in a 100 cc. volumetric flask. This tube

<sup>1</sup> W. G. Lindsay, *Columbia School of Mines Quart.* 23, 24-7 (1902).

<sup>2</sup> W. Mecklenburg and F. Rosenkränzer, *Z. anorg. Chem.* 86, 143-53 (1914).

<sup>3</sup> L. H. Almy, *J. Am. Chem. Soc.* 47, 1381-90 (1925).

<sup>4</sup> Fred H. Heath and Frank A. Lee, *J. Am. Chem. Soc.* 45, 1643-7 (1923).

preferably contains many fine holes for efficiency in absorption. Rubber fittings used must be cleaned to free them from sulfides and free sulfur. They will then show no measurable blank.

**Sample—Solids.** If the sulfide concentration is more than 25 mg. per 100 grams use a 25 gram sample. For sulfide down to about 8 mg. per 100 grams use 50 or 100 grams of sample.

**Foods.** Finely chopped samples of suitable size may be aerated according to this procedure. In general the sulfide concentration increases with the age of fish, beef or pork. Its concentration is therefore indicative of age.

**Feces.**<sup>6</sup> Add 5 per cent borax solution to the sample as a preservative until ready to use. For preparation grind the sample with 5 per cent borax solution in a ball mill to a uniform suspension. Dilute to a suitable volume with the 5 per cent borax solution. Transfer a suitable aliquot to the cylinder as sample. Use 50 per cent phosphoric acid for liberation of sulfur instead of the hydrochloric acid specified.

**Sewage and Mineral Waters.** Select the sample according to suspected sulfide content to contain, if possible, about 8 mg. of sulfide sulfur.

**Iron.**<sup>1</sup> Use 1–5 grams of pig iron drillings according to sulfur content.

**Gelatine.**<sup>8</sup> This method is for labile sulfur only, that liberated by heating with concentrated ammonium hydroxide in the presence of silver ammonium chloride.

Cut 5 grams of gelatine into pieces not over 0.635 cm. long and place in the sample container. Add 25 cc. of 1 per cent silver chloride solution prepared by solution of the silver chloride in concentrated ammonium hydroxide. Let the gelatine swell for 1 hour and dissolve by heating slowly in a beaker of water to 50°. Continue to heat for about 2 hours. Too rapid heating will cause foaming. Shake at intervals to break the skin which forms on the surface.

In 2 hours the solution should be blackened due to evolution of sulfur and its fixation as silver sulfide. Evaporate to 10 cc. Cool the solution and transfer it to the evolution flask. For this sample the evolution apparatus should be run about 1 hour, as the silver sulfide is slow in decomposing.

**Zinc Acetate Solutions—**Treat 135 grams of glacial acetic acid with

<sup>6</sup> I. St. Lorant and F. Reimann, *Biochem. Z.* 228, 300-9 (1930).

<sup>8</sup> S. E. Sheppard and J. H. Hudson, *Ind. Eng. Chem., Anal. Ed.* 4, 73-5 (1930).

a slight excess of a thick aqueous suspension of zinc oxide. Dilute to 1 liter. This contains about 20 per cent of zinc acetate. Before use filter to remove suspended solids and dilute 10 cc. to 100 cc. for a 2 per cent solution or 3 cc. to 100 cc. for a 0.6 per cent solution.

**Procedure**<sup>3</sup>—Add the sample to 50 cc. of distilled water in the cylinder described. Foaming is prevented by addition of a few drops of diphenyl ether. In the case of egg products 2 cc. of a 40 per cent solution of sodium tungstate is effective. Connect up the apparatus and sweep out the air with carbon dioxide. Let nearly all the acid flow into the cylinder from the dropping funnel. Pass carbon dioxide through at a pressure of about 40 mm. of water for 15 minutes. Shut off the carbon dioxide, disconnect the 2 receiving vessels and transfer the solution from the distilling flask into the volumetric flask. The solution and washings should amount to about 90 cc. If the solution is turbid the sulfide concentration is usually greater than that of the highest standard. In that case dilute the sample to 100 cc., mix well and take an aliquot, usually 25 cc. Add this to 15 cc. of a 2 per cent zinc acetate solution in another 100 cc. volumetric flask and dilute to about 90 cc.

To the entire sample or an aliquot as above add 5 cc. of a fresh solution of 0.04 gram of *p*-aminodimethylaniline hydrochloride in 100 cc. of 1:1 hydrochloric acid. This must not be more than 24 hours old. Mix well and add 1 cc. of 0.02 *M* acid ferric chloride solution, with gentle shaking. This contains 27 grams of ferric chloride hexahydrate in 500 cc. of concentrated hydrochloric acid diluted to 1 liter. Just before use dilute with 4 volumes of distilled water.

After 2 hours dilute to 100 cc. and compare in a colorimeter with the standard solution having a color nearest that of the unknown.

**Standard Sulfide Solutions**—Wash the hydrogen sulfide evolved from ferrous sulfide and hydrochloric or sulfuric acid with water. Pass slowly for 1 or 2 minutes into about 300 cc. of cool boiled distilled water. Add an aliquot of this to a known excess of 0.01 *N* iodine solution and titrate with 0.01 *N* sodium thiosulfate solution.

Calculate the amount of the above solution which, diluted to 500 cc., will contain 0.01 mg. of hydrogen sulfide per cc. Put into a 500 cc. volumetric flask about 435 cc. of water, 15 cc. of a 2 per cent zinc acetate solution and the volume of sulfide solution calculated. Dilute to 500 cc. and use immediately for the preparation of standards.

In each of fifteen 500 cc. volumetric flasks place 75 cc. of 2 per cent

zinc acetate solution and from 315 to 375 cc. of cool, recently boiled water. Add to these 1, 2, 3, 4, 5, 7, 10, 12.5, 15, 20, 25, 30, 40, 50, and 60 cc. portions of the standard sulfide solution. Follow at once by 25 cc. of the diamine reagent and 5 cc. of 0.02 *M* acid ferric chloride solution. The volume in each flask should be about 450 cc. before the addition of the diamine reagent. The temperature in the flasks should be uniform, not varying by more than 0.5° from that adopted for addition of reagents to the sample. Agitate gently after the addition of each reagent. After 2 hours dilute to volume. Kept in a cool, dark place, the standard solutions are unchanged for 4 or 5 weeks. At 25° the more dilute ones acquire a greenish cast in about 3 weeks.

#### HYDROGEN SULFIDE AS COLLOIDAL LEAD SULFIDE

Hydrogen sulfide may be determined in water by the brown color of colloidal lead sulfide formed.<sup>10</sup> This may be done at the point where the sample is taken. The color of the standard fades slowly due to oxidation of lead sulfide by dissolved oxygen. Samples containing hydrogen sulfide do not usually contain oxygen. The developed sample will therefore not ordinarily change on standing if protected from the air. Iron in small amounts does not interfere.

If the water is originally colored, treat a sample with the reagent and let stand until the color of lead sulfide has entirely faded. Use this for development of the standard color.

**Procedure**—Let the water to be examined for hydrogen sulfide flow through a calibrated glass-stoppered flask of about 100 cc., until the air in the flask has all been displaced. Introduce into the bottom of the flask by means of a long-stemmed pipet, 5 cc. of a reagent containing 10 grams of sodium potassium tartrate, 10 grams of ammonium chloride, and 0.1 gram of lead acetate per 100 cc. in 1:5 ammonium hydroxide. Close the flask and mix. Put 100 cc. of hydrogen sulfide-free water into a beaker and add 5 cc. of the lead reagent described. Dissolve 0.1083 gram of pure hydrated sodium sulfide in 1 cc. of a 50 per cent solution of sodium nitrate to which 1 cc. of 1:1 hydrochloric acid has been added. Dilute to 10 cc. and add from a buret to the colorless solution until the color matches that of an equal volume of the sample, transferred to a similar beaker. The sodium sulfide solution must be prepared at the time the titration is made due to its rapid oxidation by air. One gram of hydrated

---

<sup>10</sup> L. W. Winkler, *Z. anal. Chem.* 52, 641-5 (1913).



sodium sulfide corresponds to 92.26 cc. of hydrogen sulfide at 0° and 760 mm. The 0.1083 gram used corresponds to 10 cc. of hydrogen sulfide.

### SULFIDES AS ARSENIOS SULFIDE

Sulfur in basic and acid pig irons, as well as in steel, may be determined by evolution as hydrogen sulfide. The hydrogen sulfide comes into contact with paper impregnated with arsenious oxide, forming a yellow stain of arsenious sulfide.<sup>11</sup> The procedure is convenient where a great many estimations are to be made, in place of the cadmium sulfide method. Checks to about 4 per cent with the volumetric and gravimetric methods were obtained in a laboratory doing about 100 samples a day.

**Arsenious Oxide Paper**—Shake 10 grams of pure powdered arsenious oxide with 30 cc. of concentrated hydrochloric acid. Add 500 cc. of boiling water and heat until dissolved. Dilute to 1 liter. Soak filter paper such as C. S. and S. No. 598 in this and drain over glass rods. Cut the paper while moist into pieces 10 cm. square and keep in a desiccator over water.

**Procedure**—Introduce 1 gram of steel drillings or fine pig iron drillings into a slightly conical vessel. A water glass is suitable. Add 10 cc. of benzine and 50 cc. of 7:3 hydrochloric acid. Cover the vessel with moist arsenious oxide paper 10 cm. square. Place on this a piece of white felt of the same size. Cover with a piece of ebonite of the same size and weight down with a disc of lead weighing about 500 grams. The hydrogen and hydrogen sulfide evolved escape uniformly through the paper and produce a uniform tint. Do not disturb after the evolution is started as breaking up the film of benzine floating on the surface will cause irregular stains on the surface of the paper.

At the same time run a similar determination with standard steels or pig irons. Decomposition may be accomplished in an hour with finely ground drillings. Compare the stains by transmitted light.

**Standards**—Suitable standards are prepared from iron or steel of known composition and the estimation made by eye from these. The following are usually sufficient.

Steel corresponding to 0.035 and 0.065 per cent of sulfur.

Basic pig iron corresponding to 0.05, 0.12, and 0.17 per cent of sulfur.

Acid pig iron corresponding to 0.05, 0.12 and 0.17 per cent of sulfur.

---

<sup>11</sup> G. Mission, *Iron Age* 93, 1253 (1914).

## BY EVOLUTION AS HYDROGEN SULFIDE

Sulfur may be estimated by the stain which it produces on lead acetate paper.<sup>12</sup> One such method uses an apparatus very similar to that of the Gutzeit method for arsenic.<sup>13</sup> The sulfur is evolved by reaction of a sulfur-free metal with hydrochloric acid and causes a stain of varying length on a prepared strip of lead acetate paper. The method was designed for ; of sulfur in wrapping tissue but should have a much Rigid standardization of the method of developing

ic is not obtainable. Aluminum was found to be the most satisfactory of the sulfur-free metals. Copper, nickel and lead in the sample do not interfere. The method is applicable in amounts from 0.001 to 0.01 mg. Above 0.015 mg. the accuracy is impaired.

A variation of the method<sup>14</sup> uses a paper diaphragm through which the gas must pass. Hydrogen, used as carrier in the first method, is replaced by nitrogen. This is distributed uniformly through the disc by first passing through a bed of sand or glass beads. The method has been applied to analysis of raw and canned foods, water, tin coatings of cans, etc.

Precautions to avoid sulfur in the ordinary distilled water and in reagents are essential. Samples and standards must be protected from light and air. The temperature must be sufficiently low so that drops of moisture will not condense in the parts of the apparatus containing the repared paper.

The method of evolution with hydrogen is more generally applicable as reducible forms of sulfur can be estimated by that method but not by the other.

**Apparatus—Evolution with Hydrogen.** The evolution flask is a 250 lenmeyer type as shown in Figure 88. The strip is contained in riction 5 mm. from the lower end. cork stopper. The lower end of the e carries a wisp of cotton to absorb acid spray

**Evolution with Nitrogen.** The apparatus is illustrated in Figure 89. The size of disc may be varied. For amounts of sulfur of 0.008–0.08 mg. a 32 mm. tube was found satisfactory.

<sup>12</sup> A. Drushel and C. M. Elston, *Am. J. Sci.* 42, 155-8 (1916).

<sup>13</sup> C. Scherer, Jr. and W. W. Sweet, *Ind. Eng. Chem., Anal. Ed.* 4, 103-4

<sup>14</sup> E. Lachele, *Ind. Eng. Chem., Anal. Ed.* 6, 199-201 (1934).

**Test Paper**—*Evolution with Hydrogen.* Soak Whatman's No. 40 filter paper in 1 per cent lead acetate solution. Press out the excess between sheets of dry filter paper and hang in the dark to dry. When dry, cut the sheets into strips 5 mm. wide, of a convenient length, and store in stoppered tubes until required for use.

*Evolution with Nitrogen.* Soak C. S. and S. black ribbon filter paper No. 589 in a saturated neutral lead acetate solution for 1 hour. Air-dry

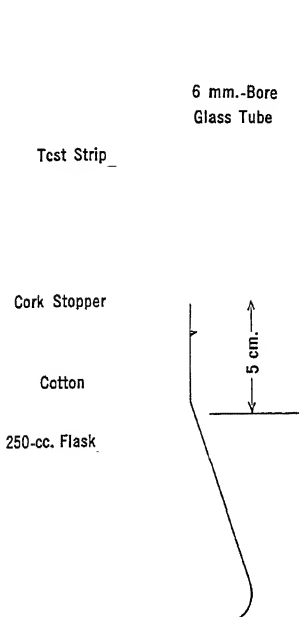


FIG. 88

Apparatus for Evolution of Sulfides  
With Hydrogen

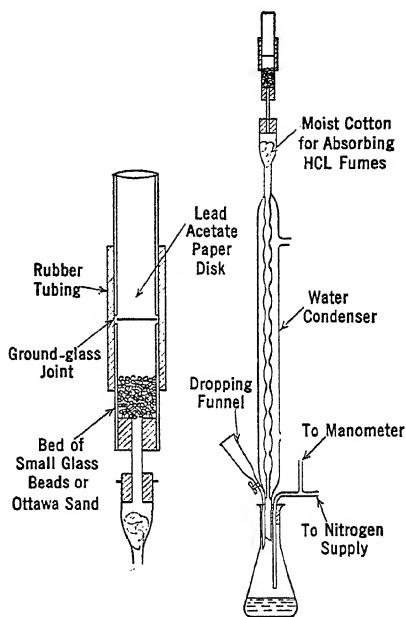


FIG. 89

Apparatus for Evolution of Sulfides  
With Nitrogen

it by waving, followed by an air blast. Cut into discs of the necessary size and store in stoppered containers. The discs will keep for several months.

**Procedure**—*Evolution with Hydrogen.* Place the sample or an aliquot of a solution in 30 cc. of 0.5 per cent sodium hydroxide solution containing 0.1 gram of hydroquinone per liter. Heat just below boiling for 10 minutes. Cool to room temperature.

Prepare aluminum reductor strips  $1.5 \times 3.0$  cm. of stock 1.0–1.5 mm.

thick. Clean just before use by immersing in boiling, 1 per cent hydrochloric acid, and rinse with distilled water. Add two reductor strips and 30 cc. of 1:2 hydrochloric acid to the sample. Stopper at once with the prepared dry tube containing a test strip. After reaction ceases, remove the strip and compare with those made to correspond to 0.001 to 0.01 mg. of sulfur.

*Evolution with Nitrogen.* Put about 100 cc. of water and the sample in the 1 liter Erlenmeyer flask of the apparatus. Displace the air with nitrogen. Add 50 cc. of 1:1 hydrochloric acid through the dropping funnel. Phosphoric or sulfuric acid may be substituted. Heat the contents of the flask to boiling while passing a stream of nitrogen at about 5 mm. pressure. Sweep out the last of the hydrogen sulfide by increasing the pressure to about 30 mm. for 2 minutes. Remove the disc and wash it in distilled water. Dry and compare with the standards. For small quantities compare by reflection, for larger quantities by the light transmitted through the stained paper.

**Standards—Evolution with Hydrogen.** Dissolve sodium thiosulfate in water and standardize at 0.1 *N* by titration with iodine. Dilute 10 cc. of this to 640 cc. Dilute 10 cc. of that solution to 1 liter. It is then equivalent to 0.001 mg. of sulfur per cc. Add 0.1 gram of hydroquinone and 5 grams of sodium hydroxide. Store in the dark. Prepare standards with 1–10 cc. of this at 1 cc. intervals. These correspond to 0.001–0.01 mg., or 0.000001–0.00001 gram. Replace these at least every 2 weeks.

*Evolution with Nitrogen.* The use of Bureau of Standards steels, such as 14b basic open hearth steel containing 0.031 per cent of volatile sulfur, is recommended as preferable to sulfide solutions. Since reducing conditions are not present, a thiosulfate standard could not be used.

#### SULFUR IN PETROLEUM DISTILLATES

The estimation of sulfur in petroleum distillates can be carried out<sup>15</sup> by the pink color of a modified Halphen test. Results agree with the lamp method and with amounts added. Hydrogen sulfide, mercaptans, sulfuric esters, organic sulfides, disulfides, sulfonic acids and sulfones do not interfere. Excessive dilution causes low results. Therefore in no case should a sample be diluted to more than 50 per cent in excess of the original volume. If the sulfur content exceeds 0.15 per cent, a

<sup>15</sup> M. K. Thornton, Jr., and J. E. Latta, *Ind. Eng. Chem., Anal. Ed.*, **4**, 441-2 (1932).

brownish precipitate will be formed which will not redissolve readily. High sulfur samples should therefore be diluted before treatment.

**Reagent**—Mix 80 cc. of refined unbleached cottonseed oil, 80 cc. of freshly distilled carbon bisulfide and 8 cc. of pyridine. The reagent must be fresh every day and should be stored in the dark in a glass-stoppered bottle.

**Procedure**—Place 20 cc. of the oil in an oil sample bottle. Add 4 cc. of reagent and heat for 30 minutes at 100°. Treat and heat standards at the same time. Also run blanks of the sample and of the naphtha in which the standards are prepared. Cool and dilute each to 25 cc. with water-white gasoline. Compare, using the Walpole technique, with the heated, untreated sample in front of the standard and the similar sample of purified naphtha in front of the sample. This corrects for natural color due to heating without reagent.

**Standards**—Purify naphtha by distillation over metallic sodium. Dissolve 1 gram of sulfur in this naphtha and dilute to 100 grams. From this prepare dilutions containing 0.010, 0.025, 0.050, 0.075 and 0.100 gram of sulfur per 100 grams. The stock solution is not stable on standing and the dilute standards may deteriorate.

### HYDROGEN SULFIDE AS SULFATE

Sulfur dioxide can be selectively oxidized from a gas by neutral potassium chlorate solution after which hydrogen sulfide can be oxidized to the sulfate and so estimated turbidimetrically.<sup>16</sup> The potassium chlorate oxidizes about 4 per cent of the hydrogen sulfide so that results tend to be low.

**Procedure**—Pass a known volume of gas through an efficient wash bottle containing 5 per cent neutral potassium chlorate solution. This removes sulfur dioxide. Then pass through a wash bottle containing 3 per cent hydrogen peroxide solution free from sulfates. Boil the peroxide solution to decompose excess peroxide, dilute to a volume which will give a proper sulfate concentration and estimate the sulfate.<sup>17</sup>

V. G. Gurevich, *J. Russ. Phys.-Chem. Soc.* 62, 111-9 (1930).

<sup>17</sup> See pp. 611-5.

## CARBON BISULFIDE BY EVOLUTION AS HYDROGEN

The method of estimation of evolution as hydrogen sulfide in extracted oils.<sup>17a</sup>

**Procedure**—Introduce 5 cc. of 1:4 hydrochloric acid and 5 cc. of oil suspected to contain carbon bisulfide into a test flask.<sup>17b</sup> Add a small piece of aluminum and warm gently to start the reaction. Complete as for other forms of sulfur.

## CARBON BISULFIDE AS COPPER XANTHATE

Carbon bisulfide may  
 . The  
 color  
 describ

**Sample**—F  
 hydroxide sol  
 then through concentrated sulfu  
 gasometer or the volume passed may be ured with a flowmeter. Then  
 it through a capillary opening in cc. of 10 per cent alcoholic  
 assium solution in a t . After the desired volume  
 gas has nse the int  
 50 cc. Nessler tube.

e sample to about 48 cc. with distilled water.  
 cent phenolphthalein solution and acetic acid  
 4 drops of 0.5 per cent cupric acetate solution,  
 mix well and dilute

As standard solution 0.0240 gram of ethyl  
 . Each . lent to 1 mg. of

hyd  
 cc. v

<sup>17a</sup> J. Navarro Sagrista, *Afinidad* 14, No. 59, 7-10 (Jan. 1934); *Chimie & Industrie* 31, 1407.

## CARBON BISULFIDE BY COPPER AND DIETHYLAMINE

The reaction of copper with sodium diethyldithiocarbamate can be used for estimation of small amounts of carbon bisulfide in the presence of copper, since carbon bisulfide and diethylamine are the equivalent<sup>19,20</sup> of the above reagent for copper. Thiophene, dimethylsulfide and ethyl mercaptan do not interfere. Thioacetic acid gives the reaction. Accuracy is good and 1 part of carbon bisulfide can be detected in 1 million parts of benzene. The method is equally applicable to toluene, carbon tetrachloride, acetone, ether and alcohol. In aqueous solution a precipitate is formed.

**Procedure**—Dilute the sample with absolute alcohol to 0.025–0.1 mg. of carbon bisulfide per cc. Pipet 1 cc. of the sample into a cylinder. Add 1 cc. of 1 per cent diethylamine in benzene or other miscible solvent. Add 1 cc. of 0.03 per cent copper acetate solution in absolute alcohol. Dilute to 10 cc. with absolute alcohol and mix. Compare with a series of standards containing 0.025, 0.050, 0.075 and 0.1 mg. of carbon bisulfide. For accurate estimation make up a new sample and standard to match at the same time and compare after 20 minutes.

**Standard**—Dissolve 1 gram of redistilled carbon bisulfide in redistilled benzene and dilute to 100 cc. Dilute 1 cc. of this to 100 cc. as a standard containing 0.1 mg. of carbon bisulfide per cc.

## SULFUR MONOCHLORIDE BY AMMONIA

In alcoholic solution ammonia liberates a violet form of sulfur from sulfur monochloride, which may then be used for its colorimetric estimation.<sup>21</sup> The method will detect 0.5 mg. of sulfur monochloride.

**Procedure**—Pipet out 5 cc. of the sample of sulfur monochloride in carbon bisulfide, carbon tetrachloride, benzene or similar nonaqueous solvent. Mix with 5 cc. of concentrated ammonium hydroxide and dilute to 100 cc. with 95 per cent alcohol. Compare with the color developed from a standard solution of sulfur monochloride in the same solvent.

<sup>19</sup> Nathaniel Tischler, *Ind. Eng. Chem., Anal. Ed.* 4, 146 (1932).

<sup>20</sup> T. Callan, J. A. Russell Henderson and N. Strafford, *J. Soc. Chem. Ind.* 51, 193-4T (1932).

<sup>21</sup> A. Castiglioni, *Ann. chim. applicata* 24, 273-7 (1934).

## CHAPTER LIX

### SELENIUM, TELLURIUM AND THIOCYANATES

#### SELENIUM BY POTASSIUM IODIDE

The use of starch t	tion
The method is appl	give
cent of selenium dioxide, or from	of from 0.0001 to 0.05 per
	m.

Add a drop of	
sample m	ith 5 cc. of 1:1
about 90 cc. Add 5 cc. of fresh	, 10 per cent potassium iodide
solution	100 compare after 5 minutes with
a stand	treat the potassium iodide solution
was added at the same time.	

d—Prepare a standard by diss  
water and diluting to 1 liter.

c. or 100 p.p.m. From this prepare a series of  
containing from 1–20 p.p.m. of selenium. If it is desired to obtain the  
results in terms of selenium dioxide, prepare the standard by dissolving  
1.000 gram of selenium dioxide per liter.

#### SELENIOUS ACID BY PYRROL

Pyrrol gives a deep blue coloration with selenious acid, suitable for  
colorimetric estimation.<sup>2</sup> In phosphoric acid without addition of ferric  
ion it is sensitive to about 2 p.p.m. By addition of iron this sensitivity  
is increased to 0.1 p.p.m. An alternative procedure replacing the phos-  
phoric acid by 1:1 sulfuric acid and ammonium molybdate renders it  
sensitive to 0.035 p.p.m. but renders it less specific. Silicic acid gives  
the same reaction. The color fades rather quickly.

(1914).  
*Festschr*



**Procedure**—To 2 cc. of sample add 1 cc. of 5 per cent ferric chloride solution and dilute to 10 cc. with syrupy phosphoric acid. Add 10 drops of a 1 per cent solution of pyrrol in 95 per cent alcohol, mix and compare with standards similarly treated at the same time.

*Alternative.* Add 1 cc. of 5 per cent ferric chloride solution to 2 cc. of sample. Add 2 cc. of 8 per cent ammonium molybdate solution and dilute to 10 cc. with 1:1 sulfuric acid. Add 10 drops of a 1 per cent solution of pyrrol in 95 per cent alcohol. Mix and compare with standards similarly treated at the same time.

#### SELENIUM BY SODIUM HYPOSULFITE

Selenious acid may be reduced with sodium hyposulfite,<sup>3</sup> resulting in a yellow to red colloidal suspension of selenium. This is used for the determination of from 0.5–5.0 mg. of selenium dioxide in a 10 cc. sample.<sup>4</sup> By concentration of a more dilute solution, as little as 0.06 mg. may be detected.

To avoid production of free hyposulfurous acid which, in the absence of selenium, will give a false color, the solution is neutralized with sodium carbonate after a few seconds. Reasonable amounts of neutral salts do not interfere. For comparison in concentrated sulfuric acid, the color is masked by the white of free sulfur precipitated. By suitable reduction of the amount of hyposulfite used the intensity of the yellow color indicates the amount of selenium present. An amount less than 0.02 mg. of selenium dioxide is not observable under these conditions.

**Procedure**—If alkaline, render the solution faintly acid with dilute mineral acid. If strongly acid make nearly neutral with sodium or ammonium hydroxide. Add 1 gram of dry sodium hyposulfite to a 10 cc. sample and shake. When the color ceases to become darker add sufficient dry sodium carbonate to render the solution faintly alkaline and compare with a series of standards similarly treated at approximately the same time.

**Standard**—Prepare a standard solution by dissolving 1.4040 grams of selenium dioxide in 1 liter of water. This corresponds to 1 mg. of selenium per cc.

<sup>3</sup> See footnote 15, p. 411.

<sup>4</sup> J. Meyer and J. Jannek, *Z. anal. Chem.* 52, 534 (1913).

## SELENIUM BY REDUCTION WITH HYDROXYLAMINE

The yellow to red color of reduced selenium<sup>5</sup> can be obtained by reduction with hydroxylamine.<sup>6</sup> Preliminary separation from interfering elements is obtained by distillation. The method with proper care will detect 1 part of selenium in 10 billion of soil, since 0.01 mg. may be estimated satisfactorily.

**Sample—Soil.** A method has been given under arsenic for separation of arsenic, selenium and germanium from interfering elements by distillation.<sup>7</sup> Dissolve the selenium precipitate by adding 10 cc. of a solution of 1 gram of bromine in 10 cc. of 45 per cent hydrobromic acid. Catch the solution in a 25 cc. volumetric flask.

*Pyrites and other Sulfides.* Follow the details under arsenic<sup>8</sup> and finish as for soil.

*Water.* Follow the details under arsenic<sup>9</sup> and finish as for soil.

*Vegetable Matter.* Follow the details under arsenic<sup>9</sup> and finish as for soil.

*Tissue.* Follow the details under arsenic<sup>9</sup> and finish as for soil.

**Procedure—**Add to the sample 1 cc. of a solution containing 5 per cent of gum arabic. Mix and pass in sulfur dioxide until the solution is decolorized. Add 0.5 gram of hydroxylamine hydrochloride and dissolve. Dilute to volume and let stand over night.

At the same time prepare a series of standards by similar treatment. Shake the standards and sample and compare in sunlight.

## SELENIUM BY MERCUROUS CHLORIDE

Selenium can be precipitated by mercurous chloride from solution containing 6–20 per cent of hydrochloric acid. At least 16 per cent is necessary for complete reaction. At 20 per cent it is possible to detect 0.0002 mg. The color of the precipitate of excess mercurous chloride

<sup>5</sup> A. Cousen, *J. Soc. Glass Tech.* 7, 303-9 (1923).

<sup>6</sup> W. O. Robinson, H. C. Dudley, K. T. Williams and Horace G. Byers, *Ind. Eng. Chem., Anal. Ed.* 6, 274-6 (1934).

<sup>7</sup> See p. 233.

<sup>8</sup> See p. 234.

<sup>9</sup> See p. 235.

then serves for its colorimetric estimation.<sup>11a</sup> Arsenic, gold, platinum palladium, tellurium and iodine interfere but can be separated.

**Sample**—*Separation of selenium, tellurium and arsenic.* Remove gold, platinum and palladium by mercurous chloride and filter. Acidify the filtrate to make it contain 20 per cent of hydrochloric acid. Add about 5 per cent of sodium bisulfite and let stand for 15 minutes. Boil gently for a few minutes to precipitate selenium. Filter, dissolve in 1:10 hydrochloric acid containing free chlorine and heat to drive off free chlorine. Use this as sample for estimation of selenium.

Remove the tellurium from the filtrate by addition of mercurous chloride, warming if necessary to hasten reaction, and use the filtrate for estimation of arsenic by raising the hydrochloric acid concentration to 30 per cent.

**Procedure**—Transfer 0.1 gram of mercurous chloride to a beaker with 1 cc. of water and 4 cc. of concentrated hydrochloric acid which has previously been boiled with mercurous chloride and left in contact with it for 24 hours. Add sufficient sample, which contains at least 20 per cent of hydrochloric acid, to form a definite color. Mix well for a few minutes and let stand. Compare with a series of standards.

The colors developed are as follows:

Mg. Selenium	Color on Mercurous Chloride
0.2	Salmon red, cold; bright red, warm
0.05	Salmon pink
0.005	Strong pinkish cream
0.002	Pink cream
0.0005	Light cream
0.0002	Faint cream

#### TELLURIUM BY MERCUROUS CHLORIDE

Like selenium, tellurium can be precipitated by mercurous chloride.<sup>11a</sup> Interference by gold, platinum, palladium, selenium and iodine can be prevented.<sup>11b</sup> The presence of 0.003 gram of copper or 0.002 gram of iron interferes. As with selenium the presence of hydrochloric acid is

<sup>11a</sup> Gordon G. Pierson, *Ind. Eng. Chem., Anal. Ed.* 6, 437-9 (1934).

<sup>11b</sup> For details see above.

necessary. In 1:4 hydrochloric acid solution 0.0005 mg. of tellurium can be detected.

**Procedure**—Follow that for selenium,<sup>11d</sup> using a selenium-free sample. The colors developed are as follows:

Mg. Tellurium	Color on Mercurous Chloride
0.2	Grayish yellow, turns grayish brown when hot
0.05	Cream yellow
0.005	Light cream yellow
0.0005	Faint cream

#### THIOCYANATE AS FERRIC THIOCYANATE

The red color of a solution of ferric thiocyanate may be used for estimation of the thiocyanate ion,<sup>12</sup> as well as for the ferric ion. Special precautions should be taken against its bleaching by sunlight.<sup>13</sup> The determination is of biological importance in gastric juice,<sup>14</sup> in which 1 p.p.m. can be detected.

**Procedure**—Filter the sample of gastric juice if necessary and dilute to 10 cc. Add 0.5 cc. of 0.1 *N* hydrochloric acid and 0.5 cc. of 1 per cent ferric chloride solution. Mix, and compare with a standard solution of potassium thiocyanate similarly treated. The standard does not keep well and must therefore be renewed frequently<sup>15</sup> or replaced by an artificial one.

**Standard**—Dissolve 0.100 gram of potassium thiocyanate in water and dilute to 1 liter. Each cc. contains 0.1 mg. of potassium thiocyanate.

#### THIOCYANATE AS COPPER PYRIDINE THIOCYANATE

A solution of a copper salt reacts with pyridine and thiocyanate to form a blue or green complex compound,  $\text{Cu}(\text{C}_5\text{H}_5\text{N})_2(\text{CNS})_2$ , copper

<sup>11d</sup> See p. 607.

<sup>12</sup> W. Autenrieth and A. Funk, *Münch, med. Wochschr.* 59, 2736 (1912).

<sup>13</sup> Georg Lockemann and Werner Ulrich, *Biochem. Z.* 243, 150-9 (1931).

<sup>14</sup> Georg Lockemann and Werner Ulrich, *Arch. Verdauungs-Krankh.* 50, 1-26 (1931).

<sup>15</sup> Carl Urbach, *Biochem. Z.* 237, 189-98 (1931).

pyridine thiocyanate.<sup>16</sup> This can be used for the estimation of very small amounts of thiocyanate.<sup>17</sup> The thiocyanate is first precipitated as the silver salt, the salt is decomposed with hydrogen sulfide, and the complex formed in the solution by the addition of copper sulfate and pyridine. The colored compound is extracted with an organic solvent. The reaction is specific. This procedure permits the determination of 0.025–0.2 mg. of thiocyanic acid. The method has been applied to the determination of thiocyanate in pure solutions with an error of  $\pm 4$  per cent. With blood and urine, recovery of added thiocyanate showed negative errors, the maximum for blood being  $-5$  per cent, and for urine  $-8$  per cent.

**Sample**<sup>17</sup>—*Blood*. Dilute 40 cc. of oxalated blood or serum with 40 cc. of water, and add 40 cc. of 20 per cent trichloroacetic acid. Mix to deproteinize, and centrifuge. Transfer 90 cc. of clear centrifugate, corresponding to 30 cc. of blood, to another centrifuge tube and precipitate with 10 cc. of *N* silver nitrate solution. Precipitation of thiocyanate is quantitative because of the other substances present, which are precipitated with silver. These help to carry down the thiocyanate. Let the tube stand for 1 hour and centrifuge. Remove the supernatant liquid. Wash the precipitate twice with water and once with alcohol.

Suspend the precipitate in 10–20 cc. of water and pass in hydrogen sulfide. Heat to boiling and break up the particles with a glass rod, continuing the hydrogen sulfide treatment until the precipitate is completely decomposed and the thiocyanate liberated. The precipitate which remains is composed of silver sulfide only. Remove the liquid from the residue and wash with water. Pass air through the combined solution and washings to remove hydrogen sulfide. Evaporate to about 3 cc., but no less.

*Normal Urine*. Normal, protein-free urine, which is not too highly colored, can be used directly. Slightly acidify from 10–20 cc. of urine and precipitate with silver nitrate solution. Continue as for blood.

*Pathological Urine*. To 45 cc. of urine in a 50 cc. volumetric flask add 4 cc. of colloidal iron oxide and 3 to 4 drops of a saturated magnesium sulfate solution. Dilute to 50 cc. with colloidal iron oxide, mix and filter. Slightly acidify 20 cc. of filtrate, corresponding to 18 cc. of urine, precipitate with silver nitrate solution and continue as for blood.

*Feces*. Rub up 10 grams of feces with water and transfer to a 100 cc. volumetric flask. Add 10 cc. of colloidal iron oxide and 1 cc. of saturated

<sup>16</sup> G. Spacu, *Bull. soc. științe cluj* 1, 284-91 (1922).

<sup>17</sup> Konrad Lang, *Biochem. Z.* 262, 14-19 (1933).

magnesium sulfate solution and dilute to volume. Shake and centrifuge. Precipitate 50 cc. of clear centrifugate with silver nitrate solution, and continue as for blood.

*Saliva and Gastric Juice.* Saliva must first be centrifuged in order to be able to measure it quantitatively. To 2–5 cc. of sample add 8 parts of water and 1 part of colloidal iron oxide. Shake and centrifuge. Precipitate an aliquot of clear centrifugate with silver nitrate solution and continue as for blood.

*Bile.* The concentration of thiocyanate in bile is about the same as that in blood. Interfering bile pigments are removed with colloidal iron oxide. To 30 cc. or more of bile add 8 parts of water and 1 part of colloidal iron oxide. Mix and centrifuge. Take an aliquot amounting to 90 per cent of the centrifugate if only 30 cc. of bile was used, or other suitable aliquot if more bile was used. Precipitate with silver nitrate solution and continue as for blood.

*Cerebrospinal Fluid.* The thiocyanate concentration is about the same as for blood. Deproteinize 45 cc. of fluid with 5 cc. of 20 per cent trichloroacetic acid. Shake and filter. Precipitate 40 cc. of filtrate with silver nitrate solution and continue as for blood.

**Procedure**—Transfer the prepared thiocyanate solution to a graduated glass-stoppered centrifuge cup of 10 cc. capacity. Wash the previous container until the washings bring the volume to 6 cc. Add 1 cc. of pyridine and 1 cc. of 10 per cent copper sulfate solution. A cornflower blue color appears. Add a further 0.5 cc. of copper sulfate solution. Extract 3 times with 1 cc. portions of bromobenzene, centrifuging after each addition. Siphon off the bromobenene extract and filter through a small filter. If the amount of thiocyanate is high, further extraction may be necessary. The united bromobenzene extract is compared with standard thiocyanate solutions, using a series of standards, each treated like the prepared sample solution. For amounts such as are found in blood, Beer's law does not hold strictly. This is why the series of standards is used. The thiocyanate content of normal blood is 0.100–0.200 mg. per 100 cc.

**Standard**—Dissolve 8.366 grams of potassium thiocyanate in water and dilute to 1 liter. Dilute 10 cc. of this solution to 1 liter. The latter contains 0.08366 mg. of potassium thiocyanate per cc., which corresponds to 0.050 mg. of thiocyanate radical per cc. Use 0.5, 1, 2, 3, 4, 5, and 6 cc. in preparing a series of standards to correspond to 0.025, 0.050, 0.075 mg. etc. of thiocyanate. Dilute each to 6 cc. and develop the color as described for the sample.

## CHAPTER LX

### SULFITES AND SULFATES

#### SULFUR TURBIDIMETRICALLY AS SUSPENDED BARIUM SULFATE

SULFATES, or sulfur oxidized to sulfate, may be determined by the turbid appearance of suspended barium sulfate. One method is conducted turbidimetrically by noting the height of the liquid in a cylinder just necessary to make invisible a flame underneath.<sup>1,2,3</sup> The various sulfur photometers can be used,<sup>4</sup> and have been modified for some purposes. Special equipment<sup>5</sup> has also been designed. Stabilizing agents such as peptone and gum ghatti are sometimes used. A simpler method compares the turbidity of the sample with that of a series of standards precipitated under similar conditions.<sup>5a</sup>

The conditions under which the barium sulfate is precipitated must be carefully standardized. The most consistent results are obtained with 1 gram barium chloride tablets pressed together without the use of a binder.<sup>6</sup> Barium chloride crystals, sized to 20–30 mesh,<sup>7</sup> and barium chloride solution are also used. The most common applications are to the estimation of sulfur as sulfate in bomb washings and to sulfate in water.

The tube used is similar in form to a Nessler tube, with the depth calibrated in cm. The candle is located about 2 inches below the tube. Variation in the size of the flame or its distance below the tube within reasonable limits does not affect the results. Small amounts of nitrates do not interfere. A large excess of hydrochloric acid causes low results.

**Sample—Water.** Unless the sulfate content is unusually high take 50 cc. as sample. If sulfate is less than 40 p.p.m. take a larger sample, evaporate to less than 75 cc. and cool.

<sup>1</sup> J. I. D. Hinds, *J. Am. Chem. Soc.* **18**, 661 (1896).

<sup>2</sup> D. D. Jackson, *Ibid.* **23**, 799 (1901).

<sup>3</sup> O. Schreiner and G. H. Failyer, *Dept. Agr., Bur. Soils Bull.* **31**, 54-5 (1906).

<sup>4</sup> K. S. Kemmerer and P. W. Boutwell, *Ind. Eng. Chem., Anal. Ed.* **4**, 423-5 (1932).

<sup>5</sup> E. Obermer and R. Milton, *J. Lab. Clin. Med.* **17**, 792-804 (1932).

<sup>5a</sup> M. V. Alekseeva, *J. Applied Chem. U.S.S.R.* **7**, 616-22 (1934).

<sup>6</sup> H. F. Muer, *J. Ind. Eng. Chem.* **3**, 553 (1911).

<sup>7</sup> Paul L. Boutwell and Edward W. Toepfer, *Ind. Eng. Chem., Anal. Ed.* **4**, 117-9 (1932).

TABLE 6. TURBIDIMETRIC SULFUR TABLE. FOR USE WITH JACKSON'S  
CANDLE TURBIDIMETER. SULFUR AND SO<sub>2</sub> CONTAINED IN  
100 CC. PRECIPITATED.

Depth cm.	S mg.	SO <sub>2</sub> mg.	Depth cm.	S mg.	SO <sub>2</sub> mg.	Depth cm.	S mg.	SO <sub>2</sub> mg.
1.0	20.0	50.0	5.0	3.66	9.15	9.0	2.30	5.75
1.1	18.0	45.0	5.1	3.60	9.00	9.1	2.28	5.70
1.2	16.5	41.3	5.2	3.54	8.85	9.2	2.26	5.65
1.3	15.0	37.5	5.3	3.49	8.73	9.3	2.25	5.63
1.4	13.5	33.8	5.4	3.43	8.58	9.4	2.23	5.58
1.5	12.5	31.3	5.5	3.38	8.45	9.5	2.21	5.53
1.6	11.2	28.0	5.6	3.33	8.33	9.6	2.19	5.48
1.7	10.0	25.0	5.7	3.28	8.20	9.7	2.18	5.45
1.8	9.5	23.8	5.8	3.24	8.10	9.8	2.16	5.40
1.9	9.0	22.5	5.9	3.20	8.00	9.9	2.15	5.38
2.0	8.5	21.3	6.0	3.15	7.88	10.0	2.13	5.33
2.1	8.0	20.0	6.1	3.11	7.78	10.1	2.11	5.28
2.2	7.6	19.0	6.2	3.07	7.68	10.2	2.10	5.25
2.3	7.3	18.3	6.3	3.03	7.58	10.3	2.09	5.23
2.4	7.0	17.5	6.4	2.99	7.48	10.4	2.07	5.18
2.5	6.7	16.8	6.5	2.95	7.38	10.5	2.06	5.15
2.6	6.5	16.3	6.6	2.92	7.30	10.6	2.04	5.10
2.7	6.3	15.8	6.7	2.88	7.20	10.7	2.03	5.08
2.8	6.1	15.3	6.8	2.85	7.13	10.8	2.02	5.05
2.9	5.9	14.8	6.9	2.82	7.05	10.9	2.01	5.03
3.0	5.7	14.3	7.0	2.79	6.98	11.0	2.00	5.00
3.1	5.5	13.8	7.1	2.76	6.90	11.1	1.98	4.95
3.2	5.4	13.5	7.2	2.73	6.83	11.2	1.97	4.93
3.3	5.2	13.0	7.3	2.70	6.75	11.3	1.95	4.88
3.4	5.1	12.8	7.4	2.67	6.68	11.4	1.94	4.85
3.5	5.0	12.5	7.5	2.64	6.60	11.5	1.93	4.83
3.6	4.85	12.25	7.6	2.61	6.53	11.6	1.92	4.80
3.7	4.75	12.00	7.7	2.59	6.48	11.7	1.91	4.78
3.8	4.63	11.75	7.8	2.56	6.40	11.8	1.90	4.75
3.9	4.52	11.50	7.9	2.54	6.35	11.9	1.89	4.73
4.0	4.43	11.25	8.0	2.51	6.28	12.0	1.88	4.70
4.1	4.33	11.00	8.1	2.49	6.23	12.1	1.87	4.68
4.2	4.24	10.75	8.2	2.47	6.18	12.2	1.86	4.65
4.3	4.16	10.50	8.3	2.44	6.10	12.3	1.85	4.63
4.4	4.08	10.25	8.4	2.42	6.05	12.4	1.84	4.60
4.5	4.00	10.00	8.5	2.40	6.00	12.5	1.83	4.58
4.6	3.93	9.83	8.6	2.38	5.95	12.6	1.82	4.55
4.7	3.86	9.65	8.7	2.36	5.90	12.7	1.81	4.53
4.8	3.79	9.48	8.8	2.34	5.85	12.8	1.80	4.50
4.9	3.72	9.30	8.9	2.32	5.80	12.9	1.79	4.48
13.0	1.78	4.45	17.1	1.49	3.73	21.1	1.24	3.10
13.1	1.77	4.43	17.2	1.49	3.73	21.2	1.23	3.08
13.2	1.76	4.40	17.3	1.48	3.70	21.3	1.23	3.08
13.3	1.75	4.38	17.4	1.47	3.68	21.4	1.22	3.05
13.4	1.74	4.35	17.5	1.47	3.68	21.5	1.21	3.03
13.5	1.73	4.33	17.6	1.46	3.65	21.6	1.21	3.03
13.6	1.73	4.33	17.7	1.45	3.63	21.7	1.20	3.00
13.7	1.72	4.30	17.8	1.44	3.60	21.8	1.20	3.00
13.8	1.71	4.28	17.9	1.44	3.60	21.9	1.19	2.98
13.9	1.70	4.25	18.0	1.43	3.58	22.0	1.18	2.95



TURBIDIMETRIC SULPHUR TABLE—*Continued*

Depth cm.	S mg.	SO <sub>2</sub> mg.	Depth cm.	S mg.	SO <sub>2</sub> mg.	Depth cm.	S mg.	SO <sub>2</sub> mg.
14.0	1.70	4.25	18.1	1.43	3.58	22.1	1.18	2.95
14.1	1.69	4.23	18.2	1.42	3.55	22.2	1.17	2.93
14.2	1.68	4.20	18.3	1.41	3.53	22.3	1.16	2.90
14.3	1.67	4.18	18.4	1.41	3.53	22.4	1.16	2.90
14.4	1.66	4.15	18.5	1.40	3.50	22.5	1.15	2.88
14.5	1.66	4.15	18.6	1.40	3.50	22.6	1.15	2.88
14.6	1.65	4.13	18.7	1.39	3.48	22.7	1.14	2.85
14.7	1.64	4.10	18.8	1.38	3.45	22.8	1.13	2.83
14.8	1.63	4.08	18.9	1.38	3.45	22.9	1.13	2.83
14.9	1.62	4.05	19.0	1.37	3.43	23.0	1.12	2.80
15.0	1.62	4.05	19.1	1.37	3.43	23.1	1.11	2.78
15.1	1.61	4.03	19.2	1.36	3.40	23.2	1.11	2.78
15.2	1.60	4.00	19.3	1.35	3.38	23.3	1.10	2.75
15.3	1.60	4.00	19.4	1.35	3.38	23.4	1.09	2.73
15.4	1.59	3.98	19.5	1.34	3.35	23.5	1.08	2.70
15.5	1.59	3.98	19.6	1.34	3.35	23.6	1.08	2.70
15.6	1.58	3.95	19.7	1.33	3.33	23.7	1.07	2.68
15.7	1.57	3.93	19.8	1.32	3.30	23.8	1.06	2.65
15.8	1.57	3.93	19.9	1.32	3.30	23.9	1.05	2.63
15.9	1.56	3.90	20.0	1.31	3.28	24.0	1.05	2.63
16.0	1.56	3.90	20.1	1.30	3.25	24.1	1.04	2.60
16.1	1.55	3.88	20.2	1.30	3.25	24.2	1.03	2.58
16.2	1.54	3.85	20.3	1.29	3.23	24.3	1.03	2.58
16.3	1.54	3.85	20.4	1.28	3.20	24.4	1.02	2.55
16.4	1.53	3.83	20.5	1.28	3.20	24.5	1.02	2.55
16.5	1.53	3.83	20.6	1.27	3.18	24.6	1.01	2.53
16.6	1.52	3.80	20.7	1.26	3.15	24.7	1.01	2.53
16.7	1.52	3.80	20.8	1.26	3.15	24.8	1.00	2.50
16.8	1.51	3.78	20.9	1.25	3.13	24.9	1.00	2.50
16.9	1.50	3.75	21.0	1.25	3.13	25.0	1.00	2.50
17.0	1.50	3.75						

## SULFATE BY THE TYNDALL EFFECT

As distinguished from the usual turbidimetric methods for sulfates the suspension may be estimated from its turbidity as measured by the Tyndall effect.<sup>17a</sup> Special equipment is required for the purpose. While the originators used it only for water it should be applicable for other sulfate solutions. No standard is needed.

The method uses 20–30 mesh barium chloride because that has been used for the Parr turbidimeter. The bulb must be exactly standardized. The calibration of the instrument is shown in Figures 90 to 93, to illustrate the nature of the curve in each range. For use such curves must be obtained for each individual instrument and bulb. There is no appreciable interference by calcium, magnesium, ferric, aluminum,

<sup>17a</sup> R. T. Sheen, H. L. Kahler and E. M. Ross, *Ind. Eng. Chem., Anal. Ed.* **7**, 262-5 (1935).

phosphate or silicate ions alone or in compatible mixtures provided a blank is run to correct for iron. The limit of 100 p.p.m. for readings can be extended by accurate dilution. Temperatures of 17–32° did not affect the result, nor did different lots of barium chloride. The method can be carried out in less than 10 minutes. Reasonable accuracy

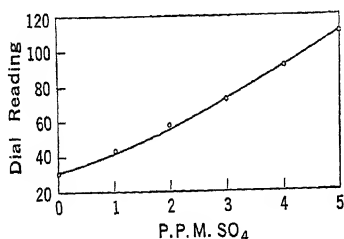


FIG. 90

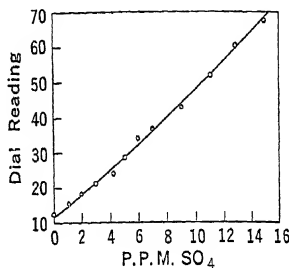


FIG. 91

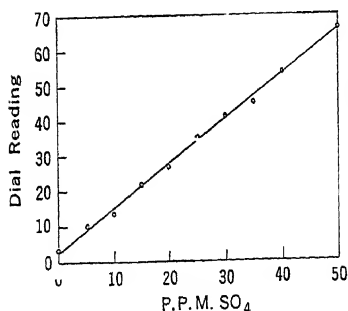


FIG. 92

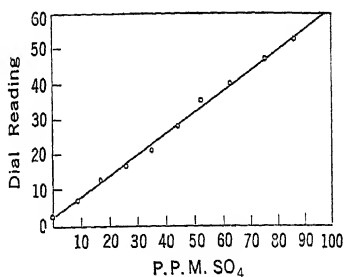


FIG. 93

FIG. 90—0.5 p.p.m. of Sulfate.

FIG. 91—0.15 p.p.m. of Sulfate.

FIG. 92—0.50 p.p.m. of Sulfate.

FIG. 93—0.100 p.p.m. of Sulfate.

Gray Filter, 20 mm. Cell, 50 cc. Sample

Milk Filter, 20 mm. Cell, 50 cc. Sample

Clear Filter, 20 mm. Cell, 50 cc. Sample

Clear Filter, 10 mm. Cell, 25 cc. Sample

#### Calibration Curves for Betz-Hellige Turbidimeter

is obtainable by even inexperienced operators, accuracy in general within 5 per cent.

used at  $T$  are 10 or 20 mm. in depth with the short plunger in place to adjust the depth used. The bottoms of this plunger and of the tubes are plano-parallel plates and are fused on. To cover different ranges of turbidity insert standard gray-glass, milk-glass or clear filter plates over the precision slit.

In use the rays from the bulb are reflected into the column and thus produce the Tyndall effect. Light rays from the bulb also pass through the calibrated slit and are reflected through the circular opening in the silver of the mirror to the ocular. Thus there is a circular spot in the center of the Tyndall effect which is lighter or darker than the Tyndall light depending on the opening of the calibrated slit. By regulating the opening of the slit the intensity of this light is varied and the Tyndall effect measured.

**Sample**—If turbid, filter or make a blank reading after addition of the standard acid-salt solution but before addition of barium chloride to precipitate barium sulfate.

**Procedure**—Prepare a standard salt-acid solution containing 240 grams of sodium chloride and 20 cc. of concentrated hydrochloric acid per liter. Filter until it shows a zero turbidity.

*Sulfate below 50 p.p.m.* Mix a 50 cc. sample with 10 cc. of the standard salt-acid solution. Add 0.29 gram of 20-30 mesh barium chloride within an accuracy of 5 per cent. This can be obtained with a standard measuring cup. Stir for exactly 1 minute and transfer to the 20 mm. cell. Take the reading 4-6 minutes after the precipitation. Average 2 readings and obtain the value from calibration curves developed for the equipment.

*Sulfate 50-100 p.p.m.* Mix 25 cc. of sample with 5 cc. of the standard acid-salt solution. Treat as for 0-50 p.p.m. but read in the 10 mm. cell.

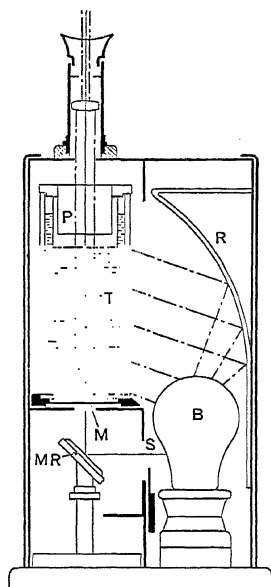


FIG. 94

Betz-Hellige Turbidimeter  
for Estimation of Sulfate  
by the Tyndall Effect

## SULFATE TURBIDIMETRICALLY IN WATER

A rough estimation may be made by a method so much simpler as to justify a separate outline.<sup>18</sup> The principal purpose is in preliminary examination of water samples in tubes.

**Procedure**—To a 10 cc.

sulfate solutions add 1  
mg 48 cc. of concentrated  
chloride per liter. Mix well and compare.

## SULFATE NEPHELOMETRICALLY AS BARIUM SULFATE

The amount of sulfate present in a solution may be estimated nephelo-

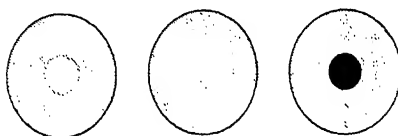


FIG. 95

Observation Fields of Betz-Hellige Turbidimeter. Center, Balanced; Left and Right, Unbalanced

metrically as barium sulfate. This comparison is made preferably in a nephelometer.<sup>19</sup> Rough comparisons can be obtained in test tubes or graduated cylinders.<sup>20</sup>

The method can, if necessary, be carried out on 15 cc. of dog or beef blood or 30 cc. of human blood with an accuracy of 5 per cent.<sup>21</sup> The same method is applicable to urine.<sup>22</sup> The amount of sulfur as sulfate per 25 cc. must be between 0.02 and 0.2 mg. for a satisfactory barium sulfate dispersion to be obtained.

Gelatine or glycerine<sup>23</sup> is used to render the suspensions more stable. Limiting concentrations of other ions which may be present are sodium 0.15 per cent, magnesium 0.003 per cent, zinc 0.006 per cent, cadmium 0.006 per cent, mercury 0.04 per cent and aluminum 0.002 per cent.

<sup>18</sup> W. D. Collins and Margaret D. Foster, *Ind. Eng. Chem.* 15, 1078-80 (1923).

<sup>19</sup> W. Denis, *J. Biol. Chem.* 49, 311-7 (1921).

<sup>20</sup> L. Lorber, *Biochem. Z.* 163, 476-79 (1925).

<sup>21</sup> W. Denis and L. Reed, *J. Biol. Chem.* 71, 191-204 (1926).

<sup>22</sup> W. Denis and L. Reed, *J. Biol. Chem.* 71, 205-8 (1926).

<sup>23</sup> J. Krepelka and A. Kalina, *Chem. Listy* 22, 545-50 (1928).

Beyond these values magnesium gives low results, the others high results. Barium phosphate tends to precipitate unless the solution is properly acid. If too acid, barium acid sulfate is formed. The optimum pH is 3.0–3.8. If nitrate is present the results will be low. A uniform salt concentration in sample and standard is essential.

**Reagents—Gelatin Solution.** To prepare a gelatin solution free from sulfates which will keep for several months, add to 50 grams of commercial gelatin, 900 cc. of approximately 0.01 *N* hydrochloric acid, and 100 cc. of a 5 per cent solution of barium chloride. Heat 1 hour in a boiling water bath, shaking occasionally. Cool, add 50 cc. of egg white and mix. Heat again for 30 minutes in boiling water, or until the egg white has coagulated and removed all traces of barium sulfate. Centrifuge while hot. Pour off the clear supernatant liquid into small wide-mouthed bottles and stopper with cotton. Sterilize by heating the bottles for an hour in boiling water, cool, and keep on ice.

**Zinc Nitrate Oxidizing Mixture.** Dissolve 25 grams of zinc nitrate, 25 grams of sodium chloride, and 10 grams of ammonium chloride in 100 cc. of distilled water. Filter through ashless paper.

**Sample—Deproteinizing blood.** To 50 cc. of citrated or oxalated blood or plasma add 110 cc. of distilled water and 40 cc. of 20 per cent trichloroacetic acid solution. Shake, let stand for 15 minutes, and centrifuge to remove the major portion of the precipitate. Filter the supernatant liquid through an ashless filter paper to give the deproteinized filtrate.

**Inorganic sulfates in dog or beef blood.** To 15 cc. of trichloroacetic acid filtrate add 2 cc. of 0.6 *N* sodium hydroxide solution and 1 cc. of 5 per cent gelatin solution.

**Inorganic sulfates in human blood.** To 25 cc. of trichloroacetic acid filtrate add 4 cc. of 0.6 *N* sodium hydroxide solution and 1 cc. of 5 per cent gelatin solution. If the sulfate is found to be high repeat according to the procedure for dog or beef blood.

**Total sulfates in dog or beef blood.** To 10 cc. of blood filtrate add 4 cc. of *N* hydrochloric acid and evaporate at such a rate that solid particles begin to settle out in not less than 15 to 20 minutes. Evaporate the last few cc. carefully to avoid discoloration. To the residue add 15 cc. of water, 2 cc. of 0.6 *N* sodium hydroxide solution and 1 cc. of 5 per cent gelatin solution.

**Total sulfates in human blood.** Use the same method as for dog or

beef blood except take 15 cc. of blood filtrate instead of 10 cc. The sample may be decreased to 5 cc. or increased to 15 cc.

*Total sulfur in dog or beef blood.* To 3–5 cc. of blood add 1 cc. of zinc nitrate oxidizing mixture. Evaporate to dryness in a large Pyrex tube and heat until no more fumes are evolved. If any nitrates are present they will be obtained. Dissolve in 2 cc. of *N* hydrochloric acid.

solution.

as above, using from 5–10 cc.

of

may be used.

*Inorganic sulfates in urine.* Dilute 0.1 mg. per cc. of sulfur as sulfate. Human urine 1:10 or 1:5, dog urine 1:10. To 1 cc. of diluted urine add 1 cc. of hydrochloric acid, 2 cc. of 0.6 *N* sodium hydroxide solution and 5 per cent sulfate-free gelatin solution.

*Total sulfates in urine.* Place 1 cc. of diluted urine in a Pyrex tube with 4 cc. of *N* hydrochloric acid, and boil gently for 15 to 20 minutes. Discontinue heating when solid particles appear. Dissolve in 15 cc. of 0.1 *N* hydrochloric acid. Add 2 cc. of 0.6 *N* sodium hydroxide solution and 5 per cent gelatin solution. To 1 cc.

of  
hydrochloric acid. Add 15 cc. of

the difference between total sulfur

this method is suitable up to 30 mg. of sulfate radical per liter.

containing 1 cc. of potassium sulfate solution 10 mg. per cc. For comparison with a standard solution of hydrochloric acid to the same

sulfur. This will obviate repetition of many determinations when abnormally low sulfur values occur.

To the prepared sample and standard add 5 cc. of a 1 per cent barium chloride solution, mix well and let stand for 15 minutes. Compare in the nephelometer. Only a standard prepared at the same time in the same way may be used for comparison.

**Standard**—Dissolve 0.5437 gram of potassium sulfate in water and dilute to 1 liter. Each cc. is equivalent to 0.1 mg. of sulfur.

#### SULFATE SEPARATED AS BENZIDINE SULFATE BY IODINE AND POTASSIUM IODIDE

Sulfate may be precipitated with benzidine hydrochloride.<sup>25,26</sup> If this precipitate is dissolved in water and treated with a mixture of iodine, potassium iodide, and ammonium hydroxide a brown color is developed. This may be compared colorimetrically with a standard, with accuracy to about 2 per cent.

**Sample**—*Urine, albumin absent.* Pipet 2 cc. of adult's urine, 10 cc. of bottle-fed infant's urine, or 20 cc. of breast-fed infant's urine into a 50 cc. volumetric flask. Add a volume of 95 per cent alcohol equal to one-third the volume of urine taken. Add 1 cc. of a 0.33 per cent solution of uranium acetate and 1 cc. of an acetate solution containing 100 grams of sodium acetate and 30 cc. of glacial acetic acid per liter of solution. Shake, dilute to volume, and filter.

Prepare a saturated alcoholic solution of benzidine by shaking 1 gram of benzidine sulfate with a liter of 95 per cent alcohol. Let stand overnight and filter. Put 1 cc. of urine filtrate into a centrifuge tube and add 5 cc. of the prepared benzidine reagent. Stir with a glass rod a few minutes. Centrifuge and wash the precipitate 2 or 3 times with the benzidine solution.

*Urine, albumin present.* If the urine contains albumin, it must be removed before the precipitation of sulfate. To the sample add 1 cc. of the uranium acetate solution and 1 cc. of the sodium acetate-acetic acid solution described above. Add 95 per cent alcohol equal in volume to one-third that of the urine.

<sup>25</sup> C. K. Fiske, *J. Biol. Chem.* **47**, 59 (1921).

<sup>26</sup> S. Yoshimatsu, *Tôhoku J. Exptl. Med.* **7**, 119-24, 553-9 (1926).

Prepare an alumina cream<sup>27,28</sup> by treating a 1 per cent solution of ammonium alum with a slight excess of a 1:10 solution of ammonium hydroxide. Wash the precipitated cream by decantation until the wash water shows only a faint trace of residue on evaporation. Dilute the sample to volume with this cream. Shake and filter. To 1 cc. of filtrate add 5 cc. of benzidine reagent and proceed as above.

**Blood.** To 10 cc. of citrated or oxalated blood or plasma in a 50 cc. volumetric flask add 1 cc. of uranium acetate solution and 1 cc. of sodium acetate-acetic acid solution, 13 cc. of alcohol and 15 cc. of water. Dip into boiling water and shake until the protein coagulates. Cool and dilute to volume with alumina cream. Filter, transfer 5 cc. of filtrate to a centrifuge tube, add 5 cc. of benzidine reagent and proceed as for urine.

**Milk.** Put 25 cc. of breast milk or 10 cc. of cow's milk into a 50 cc. volumetric flask, add 1 cc. of uranium acetate solution, 1 cc. of sodium acetate-acetic acid solution, and 10 cc. of alcohol. Shake in boiling water until coagulation occurs. Dilute to volume with alumina cream, prepared as described under urine. Filter and put 5 or 10 cc. of filtrate into a centrifuge tube. Add an equal volume of benzidine reagent, stir a few minutes and let stand for half an hour. Centrifuge, and proceed as for urine.

**Iodine Mixture**—Mix 2 volumes of a solution containing 0.1 gram of iodine and 0.2 gram of potassium iodide in 300 cc. of water, with 1 volume of 1:4 ammonium hydroxide.

**Procedure**—Transfer the precipitate from the centrifuge tube to a 100 cc. volumetric flask and dissolve in about 80 cc. of water. In another 100 cc. flask put 10 cc. of a standard solution of benzidine sulfate and add about 70 cc. of water. To each flask add 10 cc. of the iodine mixture, let stand a minute, dilute to 100 cc. and compare at once by balancing.

**Standard**—Dissolve 0.0705 gram of pure benzidine sulfate in water and dilute to 2 liters. Each cc. corresponds to 0.01 mg. of sulfur trioxide. This solution is stable for 6 months.

#### SULFATE SEPARATED AS BENZIDINE SULFATE, BY FURFUROL

Sulfate separated as benzidine sulfate may be estimated from the

<sup>27</sup> J. Marshall and W. H. Welker, *J. Am. Chem. Soc.* 35, 820-2 (1913).

<sup>28</sup> G. Tracy and W. H. Welker, *J. Biol. Chem.* 22, 55-7 (1915).



yellow color produced with furfural.<sup>29</sup> The method may be applied to the benzidine washings or more accurately but less conveniently, to the precipitate of benzidine sulfate. The method has been applied mainly to urine analysis. Considerable chloride ion causes low results. Phosphoric acid causes high results.

**Sample**—Clarify the solution as in the previous method.

**Procedure**—To 5 cc. of solution in a test tube add 2 drops of a 0.04 per cent solution of bromophenol blue. Add *N* hydrochloric acid until the indicator turns a pure yellow color. Then add 5 cc. of water and 2 cc. of 5 per cent benzidine hydrochloride solution. Add 5 cc. of 90 per cent acetone to insure complete precipitation. Filter and wash 3 times with 90 per cent acetone.

Dissolve the precipitate in 15 cc. of a mixture of equal volumes of 0.04 *N* sodium hydroxide solution and 95 per cent alcohol. Add 1 cc. of 5 per cent barium chloride solution, mix well and make acid to phenolphthalein with *N* hydrochloric acid. Add 0.5 cc. in excess and dilute to 20 cc.

Take 5 cc. of the above solution for development of color. As standard take a suitable amount of benzidine sulfate solution diluted to 5 cc. with 0.025 *N* hydrochloric acid. To sample and standard add 2 cc. of 1 per cent aqueous solution of furfural. Mix and compare at once.

#### SULFATE SEPARATED AS BENZIDINE SULFATE, DIAZOTIZED AND COUPLED

After separating as benzidine sulfate another indirect method is to diazotize and couple with phenol to give a yellow dye.<sup>30</sup> Sufficient acid is used to prevent precipitation of phosphates. Benzidine sulfate is insoluble in the 50 per cent alcohol used for washing. It does not pack tightly so that decantation is difficult. The method has been worked out for urine but should have a much wider applicability. About 0.1 mg. of sulfur should be present in the sample.

**Sample**—Filter the sample and pipet 5 cc. into a 10 cc. cylinder. Acidify with acetic acid, if necessary, and dilute to 10 cc. To 2 cc. of the diluted urine add 2 cc. of a benzidine hydrochloride solution. Prepare this by dissolving 4 grams of benzidine hydrochloride and 10 cc. of

<sup>29</sup> Junzo Yamazaki, *Bull. Chem. Soc. Japan* 3, 173-80 (1928).

<sup>30</sup> B. S. Kahn and S. L. Leiboff, *J. Biol. Chem.* 80, 623-9 (1928).

concentrated hydrochloric acid in 500 cc. of water and filtering. If necessary decolorize the reagent with activated carbon. Add 4 cc. of 95 per cent alcohol to sample and reagent and mix. Let stand 15 minutes and centrifuge. Decant the upper layer and dry the edge of the tube with filter paper. Mix the precipitate with 5 cc. of 50 per cent alcohol, centrifuge and decant. Wash again in the same way.

To the precipitate add 0.5 cc. of concentrated hydrochloric acid and 5 cc. of water in several portions, mixing well. Cool, add 1 cc. of 10 per cent sodium nitrate solution and let stand 5 minutes. Transfer to a cylinder and rinse the centrifuge tube twice with 10 cc. portions of water.

Treat 2 cc. of standard ammonium sulfate the same as the 2 cc. of diluted urine. Carry through the stage of diazotizing and transfer in the same way as the sample.

**Procedure**—Dissolve 50 grams of colorless phenol crystals in 500 cc. of water, add 15 per cent sodium hydroxide solution until just alkaline to litmus and dilute to 1 liter. Add 5 cc. of 15 per cent sodium hydroxide solution and 5 cc. of the sodium phenolate solution to sample and standard. Dilute each to 100 cc. and compare by dilution.

**Standard Sulfate Solution**—Weigh 4.1216 grams of pure dry ammonium sulfate. Dissolve in water and dilute to 1 liter. Each cc. contains 1 mg. of sulfur. Dilute 50 cc. to 1 liter for use, this being equivalent to 0.05 mg. of sulfur per cc. Add a few drops of chloroform to the standards as preservative.

#### SULFATE BY LIBERATION OF CHROMATE AND ESTIMATION WITH DIPHENYLCARBAZIDE

By reaction of sulfate with barium chromate the corresponding amount of barium sulfate is precipitated and a soluble chromate left in solution. After removal of excess barium chromate by treatment with lime, the chromate is most satisfactorily estimated by its reddish-violet color with diphenylcarbazide.<sup>31</sup> One-tenth the amount which can be estimated by benzidine is thus determined.

Phosphates react the same as sulfates and must be removed. Some reducing substances in urine tend to reduce the chromate.<sup>32</sup> A preliminary treatment is therefore necessary. By using an amount of

<sup>31</sup> Konrad Lang, *Biochem. Z.* 213, 469-74 (1929).

<sup>32</sup> Sergius Morgulis and Martha G. Hemphill, *Biochem. Z.* 249, 409-11 (1932).

barium chromate such that between 20 per cent and 80 per cent of it will react, the method is accurate to 2 per cent. Iron interferes, but in the presence of more than 0.17 mg. of iron per cc. of final solution this is avoided by addition of 1 cc. of 20 per cent hydrochloric acid to the solution before developing the color.

**Reagents—Barium Chromate.** Dissolve 24.4 grams of barium chloride and 14.7 grams of potassium dichromate in water. Heat to boiling and mix. Filter the precipitate and boil several times with 1 per cent acetic acid solution. Wash until the wash water gives only a trace of color with diphenylcarbazine reagent and dry at 110°. Dissolve 1.2670 grams of barium chromate in 100 cc. of *N* hydrochloric acid and dilute to 1 liter. This solution is 0.01 *N*.

**Diphenylcarbazine.** Heat 6 grams of urea and 21.6 grams of phenylhydrazine for 45 minutes in a flask over a free flame. Let cool and break up the resulting mass. Extract excess phenylhydrazine with ether. Recrystallize the dried crystals from 300 cc. of boiling water. Filter and dry. Dissolve 2 grams of crystals in 10 cc. of glacial acetic acid and dilute to 100 cc. with 96 per cent alcohol.

**Sample—Preliminary Treatment of Urine.**<sup>33</sup> Add 0.5 gram of calcium hydroxide to 10 cc. of urine and shake to precipitate phosphates. Centrifuge and pipet out 2 cc. Add 5 to 10 drops of 80 per cent hydrogen peroxide and 1 drop of 1 per cent ferric chloride solution. Boil for 10 minutes to oxidize uric acid. Add 0.05 gram of manganese dioxide to destroy excess of hydrogen peroxide. Filter, after adding a drop or two of *N* sodium hydroxide solution, if necessary.

**Free Sulfate in Urine.** To 1 cc. of pretreated sample add 9 cc. of the barium chromate reagent. Mix and add 0.5 gram of calcium hydroxide. Mix and let stand for 5 minutes. Centrifuge and use 1 cc. of the clear liquid as sample.

**Total Sulfate in Urine.** Mix 1 cc. of pretreated urine, 1 cc. of water and 5 cc. of 5 per cent hydrochloric acid. Boil for 1 hour on a water bath. Cool and dilute to 10 cc. with the barium chromate reagent. Mix 0.5 gram of calcium hydroxide with this and let stand for 5 minutes. Use 1 cc. of the clear liquid as sample.

**Total Sulfur in Serum or Urine.** Add 2 cc. of fuming nitric acid to a 0.5 cc. of pretreated sample in a 10 cc. volumetric flask. Heat over

<sup>33</sup> Sergius Morgulis and Martha G. Hemphill, *J. Biol. Chem.* 96, 573-83 (1932),

a free flame and add 30 per cent hydrogen peroxide, drop by drop, until digestion is complete. When the solution is clear, evaporate to dryness. Add barium chromate solution up to 10 cc. No blue color should appear, which, if present, would show that destruction of the hydrogen peroxide was incomplete. Add 0.5 gram of calcium hydroxide, mix and let stand for 5 minutes. Use 1 cc. of the clear liquid as sample.

**Procedure**—Dilute the sample with water to 10 cc. Add 1 cc. of the reagent and compare with a standard after 20 minutes.

As standard use 0.5 cc. of the 0.01 *N* barium chromate solution with 10.5 cc. of water. Each cc. of the barium chromate solution is equivalent to 0.1603 gram of sulfur.

#### SULFATE AS THE EQUIVALENT ALKALI CHROMATE

Since barium sulfate is more insoluble than barium chromate, by interchange of radicals the alkali sulfate can be converted to alkali chromate and estimation made by the yellow color of the chromate radical.<sup>34</sup>

**Procedure**—Heat 50 cc. of sample solution to boiling. Add 1 cc. of a 1 per cent solution of barium chromate in 1:1 hydrochloric acid. Add powdered calcium carbonate until no further reaction occurs. Filter and wash the precipitate well with hot water. When cool, dilute the filtrate to 100 cc. and compare with the result of similar treatment of diluted sulfate standards.<sup>34a</sup>

#### SULFATE BY LEAD SULFIDE

A method by which sulfate in water is precipitated as lead sulfate permits estimation of the combined lead as the sulfide.<sup>34b</sup> The method should be more broadly applicable with a reasonable degree of accuracy.

**Procedure**—Transfer 5–10 cc. of sample solution to a centrifuge tube. Acidify with 2–3 drops of glacial acetic acid. Mix and add 3 cc. of 95 per cent alcohol. Add 1 cc. of 1 per cent lead nitrate solution drop by drop. Mix and centrifuge to separate lead sulfate. Wash the precipitate

<sup>34</sup> P. Guarnieri, *Ind. ital. cons. alim.* 3, 161 (1930); *Chimie et Industrie* 24, 814 (1931).

<sup>34a</sup> See pp. 186-93.

<sup>34b</sup> D. B. Iokhel'son, *Ukrain. Khim. Zhur.* 9, Wiss. Teil 25-8 (1934).

3 times with 5 cc. portions of 30 per cent alcohol to remove lead nitrate. The last washing should give no test for lead with the sulfide reagent prepared by mixing 25 cc. of 20 per cent aqueous sodium sulfide with 25 cc. of glycerol.

Dissolve the precipitate of lead sulfide in 10 cc. of 0.5 per cent sodium hydroxide solution and transfer to a Nessler tube of suitable size. Dilute almost to the mark and add 1 cc. of the sulfide reagent. Dilute to volume and mix.

To another cylinder add 1 cc. of the standard, 10 cc. of 0.5 per cent sodium hydroxide solution and dilute almost to the same volume as the sample. Add 1 cc. of the sulfide reagent, dilute to volume and mix. Compare the sample and standard by balancing.

**Standard**—Dissolve 0.8275 gram of lead nitrate, dried at 120°, in water and dilute to 1 liter. Each cc. is equivalent to 2 mg. of sulfur trioxide.

#### SULFUR DIOXIDE AS SULFATE

Potassium chlorate oxidizes sulfur dioxide quantitatively to sulfuric acid under properly controlled conditions.<sup>35</sup> Under the same conditions about 4 per cent of the hydrogen sulfide present in the gases is oxidized and therefore tends to give high results.

**Procedure**—Prepare a neutral 5 per cent solution of potassium chlorate. Pass a known volume of gas containing sulfur dioxide through a suitable wash bottle containing this solution. Dilute to a volume which gives a proper sulfate concentration for estimation of the sulfur. Determine the sulfate sulfur present by one of the preceding methods.

#### SULFUR DIOXIDE BY REDUCTION OF PHOSPHOMOLYBDIC ACID

Sulfurous acid reduces phosphomolybdic acid to a blue color suitable for colorimetric estimation.<sup>36</sup> That combined with aldehydes or ketones is not liberated by addition of phosphoric acid and cannot be estimated by this method. Sugars, alcohols, organic acids and salts do not interfere. Tannin and phenolic compounds also reduce the reagent and, if volatile, must be absent from the sample used.

<sup>35</sup> V. G. Gurevich, *J. Russ. Phys.-Chem. Soc.* **62**, 111-9 (1930).

<sup>36</sup> R. Sasaki, *Bull. Agr. Chem. Soc. Japan* **4**, 38-40 (1928).

**Sample**—Place 10–50 grams of sample in a 500 cc. distilling flask. Add 100 cc. of water, neutralize with syrupy phosphoric acid if alkaline, and add 1 cc. of excess acid. Pass a stream of carbon dioxide through the flask and through a wash bottle containing 10 cc. of 1:9 ammonium hydroxide. Heat the sample to boiling and distil at the rate of 1 cc. r minute for 1 hour.

Alternatively, omit the stream of carbon dioxide, increase the amount of acid added to the sample to 2.5 cc. and just before starting the distillation add 10 cc. of saturated sodium bicarbonate solution to the sample.

**Procedure**—Add 5 cc. of concentrated ammonium hydroxide and 5 cc. of 5 per cent phosphomolybdic acid solution to the distillate. Mix well, transfer to a 100 cc. volumetric flask and dilute to volume. Mix and compare after 20 minutes with 1 cc. of standard sulfite solution similarly treated with reagents at the same time.

**Standard**—Dissolve 1.9682 grams of crystallized sodium sulfite,  $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$ , in water and dilute to 1 liter. Each cc. contains 0.5 mg. of sulfur dioxide.

## CHAPTER LXI

### NITRATES

#### NITRATES BY PHENOLDISULFONIC ACID

SOLUTIONS containing nitrates give a yellow color<sup>1</sup> with a reagent produced by treating phenol with fuming sulfuric acid. The color is intensified when the solution is made alkaline. The reagent<sup>2</sup> is the 1, 2, 4-phenoldisulfonic acid. The ortho- and para-phenolmonosulfonic acids with nitrates give a dark green solution with no yellow color. The ortho-acid reacts in the cold, the para-acid only when heated. The phenol disulfonic acid reagent reacts either hot or cold to give the yellow color.

The color compared is due to the formation of the tripotassium salt of nitrophenoldisulfonic acid,<sup>3</sup> or by a modified procedure to the corresponding ammonium salt. Chlorides present up to 30 p.p.m.<sup>4</sup> introduce an error amounting to not over 10 per cent and are not removed in some estimations, notably water analysis.

For the highest accuracy<sup>5</sup> chlorides, carbonates, organic matter and large concentrations of nitrites must be removed. The reagent must be free from mono-acids and of reproducible composition and concentration.

This method is used because of its simplicity, but has the objections<sup>6</sup> of possible difficulty in obtaining a clear and colorless extract, possible loss of nitrates in evaporation and occasional difficulty in comparison with the series of standards due to interfering tints. Accuracy to 5 per cent in comparison is to be expected.<sup>7</sup>

**Sample—Water.** The alkalinity, chlorides, nitrites and color should have been determined. If nitrite nitrogen exceeds 1 p.p.m. error will be introduced unless it is removed. To convert nitrites to nitrates add a

<sup>1</sup> Hermann Sprengels, *Ann. der Physik u. Chemie* 121, 188 (1864).

<sup>2</sup> E. M. Chamot and D. S. Pratt, *J. Am. Chem. Soc.* 31, 922-8 (1909).

<sup>3</sup> E. M. Chamot and D. S. Pratt, *J. Am. Chem. Soc.* 32, 630 (1910).

<sup>4</sup> R. Stewart and J. E. Greaves, *J. Am. Chem. Soc.* 32, 756 (1910).

<sup>5</sup> E. M. Chamot, D. S. Pratt and H. W. Redfield, *J. Am. Chem. Soc.* 33, 366-84 (1911).

<sup>6</sup> H. J. Harper, *Ind. Eng. Chem.* 16, 180-3 (1924).

<sup>7</sup> G. S. Fraps and A. J. Sterges, *Tex. Agr. Expt. Sta., Bull.* 439, 5-22 (1931).

few drops of hydrogen peroxide free from nitrate, to the sample and heat, repeating the addition several times. As an alternative method of removing nitrites add 0.1 per cent potassium permanganate solution until a faint pink coloration persists. The value of the nitrites so oxidized to nitrates must later be allowed for.

If the nitrate nitrogen is less than 8 p.p.m. measure 50 cc. as sample. If very low 100 cc. should be used. If the nitrate nitrogen is more than 8 p.p.m. use only 1.0 cc. as sample. Add sufficient 0.02 *N* sulfuric acid to render nearly neutral.

If chlorides are below 30 p.p.m. they need not be removed although they may cause up to 10 per cent error. If above that amount add sufficient 0.4397 per cent silver sulfate solution, equivalent to 1 mg. of chloride per cc., to precipitate all but 0.1 mg. of chloride. Add a suitable amount of alumina cream, stir and allow to stand for about 10 minutes. The amount required is similar to that for decolorizing a solution of caramel of equivalent color.<sup>8,9</sup> Filter and wash well with distilled water. Evaporate just to dryness in a 3 inch porcelain evaporating dish.

*Soil, Chloride Content under 15 p.p.m.*<sup>6</sup> Dry and pulverize. Mix the soil by passing through an 8 mesh sieve. If necessary, as with soil containing small hard granules, grind so that it will pass a 60 mesh sieve. This facilitates wetting and extraction. Put 50 grams of soil or 25 grams of peat in a wide-mouth bottle and shake for 10 minutes with 250 cc. of a solution containing 5 cc. of a *N* copper sulfate solution.<sup>11</sup> Noyes<sup>12</sup> has found that a water-soil ratio of 2:1 is as efficient in extraction of nitrates as that of 8:1. If the soil is not very acid, and does not give a colored extract, add 0.4 gram of calcium hydroxide and 1 gram of magnesium carbonate. Shake for 5 minutes to precipitate the copper and iron and filter through a coarse dry filter paper. Discard the first 20 cc. Refilter, if necessary, through the same paper. A fine filter paper will retain sufficient nitrate to affect the results appreciably.<sup>8</sup>

If the soil is very acid or gives a colored extract, let settle after the first shaking and decant about 125 cc. Add to this 0.2 gram of calcium hydroxide and 0.5 gram of magnesium carbonate to precipitate copper. Shake for 5 minutes and filter.

Take 10 cc. aliquots of the treated and filtered sample if the nitrate

<sup>8</sup> C. T. Gimington and R. H. Carter, *J. Agr. Sci.* 13, 60-2 (1923).

<sup>9</sup> See pp. 630-1.

<sup>11</sup> Firman E. Bear and Robert M. Salter, *W. Va. Agr. Exp. Sta., Bull.* 159, 23 (1916).

<sup>12</sup> H. A. Noyes, *J. Ind. Eng. Chem.* 11, 213-18 (1919).



content is more than 10 p.p.m. It should not exceed 50 p.p.m. If less than 10 p.p.m. take 25 cc. aliquots. Transfer to 3-inch porcelain evaporating dishes. Evaporate to dryness on water baths. Davis finds that keeping the solutions alkaline during evaporation prevents loss of nitrates.<sup>14</sup> Gericke<sup>15</sup> finds that loss does not occur in evaporation of the aqueous solution to dryness, but in addition of phenoldisulfonic acid. He completes his evaporation at not over 70°, which may be the cause of this difference of opinion.

*Soil, Chloride Content 15-80 p.p.m.* Soils of the humid region seldom contain over 15 p.p.m. of chlorides. If the content of the sample is over that amount add 10 cc. of a 0.4 per cent solution of silver sulfate to the solution used for extraction as above. This is sufficient to remove up to 80 p.p.m. of chlorides in the original soil.

Shake for 10 minutes, let settle and decant 125 cc. Proceed with the sample as with acid solutions or colored extracts. The excess silver is removed with the copper and iron.

*Soil, Chloride Content over 80 p.p.m.* Add a sufficient amount of solid silver sulfate to the soil suspension before filtering so that all of the chloride will be precipitated. Shake for 10 minutes, let settle and decant 125 cc. Proceed with the sample as with acid soils or colored extracts. The excess silver is removed with the copper and iron.

*Soils with strongly colored extracts.* If the usual copper treatment is not sufficient add 1 gram of high grade carbon black to each 100 cc. of supernatant liquid. Shake 15 to 20 minutes before adding calcium hydroxide. If necessary add an additional 5 cc. of *N* copper sulfate to assist in complete removal of the carbon black. Proceed as for other acid soils. It is to be expected that some removal of nitrate would occur with this method and that the results will be low.

*Soils with strongly colored extracts. Alternative Method.*<sup>16</sup> If soil solutions contain a large amount of soluble organic matter they may be decolorized by alumina cream.

Shake 100 grams of soil and 5 grams of precipitated calcium carbonate with 400 cc. of distilled water for 15 minutes in a 1 liter bottle. Let settle and pipet off a 100 cc. aliquot. In a similar container put 100 cc. of distilled water. Add a 1 per cent solution of caramel prepared as for matching the color of sugar solutions until the color of this blank matches the sample. For each cc. of caramel solution used add the alumina cream

<sup>14</sup> C. W. Davis, *J. Ind. Eng. Chem.* 9, 290-4 (1917).

<sup>15</sup> W. F. Gericke, *J. Ind. Eng. Chem.* 9, 585-6 (1917).

Emerson, *Soil Science* 12, 413 (1921).

from 0.75 gram of potash alum to the sample. Shake thoroughly and filter through coarse filter paper. Proceed with aliquots as with acid soils or colored extracts.

*Fresh Plant Materials.*<sup>17</sup> Dry and grind the sample very fine. Suspend in distilled water and heat on a water bath for 30 minutes. Render the solution slightly ammoniacal and decolorize with alumina cream. Select a suitable aliquot and evaporate to dryness in a porcelain evaporating dish.

*Organic Samples.* A method has been developed by which both carbon and nitrogen can be determined in a sample.<sup>18</sup> The preparation of the sample is largely described under the method for carbon.<sup>19</sup>

Boil the contents of the flask, in which nitrogen is now present as nitric acid. This expels free chlorine. Do not evaporate below 50 cc. Dilute to a known volume and take an aliquot equivalent to not less than 0.25 mg. of nitrate nitrogen. To this hot solution add 0.05–0.1 gram of silver sulfate and shake.<sup>20</sup> This should be sufficient to precipitate all chlorides. Add 0.5–1.0 gram of calcium hydroxide and shake. Let stand 15 minutes with occasional shaking. Filter and pour the filtrate back until it comes through clear. Wash the filter with 0.5 cc. of water. Evaporate the filtrate and washings to dryness on a water bath.

*Oxides of Nitrogen.*<sup>21</sup> Prepare evacuated tubes drawn to a capillary tip and marked with a file. To sample, break off the tip. To retain the sample, fill the opening with wax.

For analysis break off the neck below the capillary portion and wax plug, under mercury. Take a sample for the usual analyses for carbon dioxide, oxygen, carbon monoxide and hydrogen. Lower the sample tube until the level of mercury inside and outside is the same. Mark this and later measure the volume to this level as the sample taken for oxides of nitrogen. Remove the tube from the mercury, allowing air to enter but avoiding loss of the gas already present.

Add 5 cc. of 10 per cent sodium hydroxide solution and 5 cc. of 3 per cent hydrogen peroxide. Close the opening with a rubber stopper. Rotate to coat the inside with a film of the solution and let stand for 30 minutes. Open, rinse the contents into a filter and collect the filtrate in a 150 cc.

<sup>17</sup> H. Shmuk, *Nauk. Agron. Zhur.* 1, 562 (1924).

<sup>18</sup> E. M. Emmert, *J. Assoc. Official Agr. Chem.* 16, 424-7 (1933).

<sup>19</sup> See pp. 104-6.

<sup>20</sup> E. M. Emmert, *J. Assoc. Official Agr. Chem.* 12, 240-7 (1929); *Ibid.* 13, 146-8 (1930).

<sup>21</sup> V. C. Allison, W. L. Parker and G. W. Jones, *Bur. Mines Tech. Paper* 249, 7-12 (1921).

beaker or suitable evaporating dish. This evaporation is carried out with a watch glass cover and a slow stream of dry air free from oxides of nitrogen passing over. Evaporate just to dryness and proceed as is usual for nitrates. By this method as low as 10 p.p.m. of oxides of nitrogen other than nitrous oxide can be determined with an accuracy to 5 p.p.m.

**Phenoldisulfonic Acid Reagent**<sup>5</sup>—Dissolve 25 grams of colorless phenol in 150 cc. of pure concentrated sulfuric acid. A trace of nitric acid present in the sulfuric acid can be removed by agitating with mercury.<sup>23</sup> Add 75 cc. of fuming sulfuric acid containing 13 per cent of free sulfur trioxide. Stir well and heat in a flask for 2 hours on a boiling water bath. The reagent so prepared is free from mono- and trisulfonic acids and may be heated in contact with a water residue for hours without development of interfering colors. Sulfonation is usually complete in one-half to 1 hour. Longer heating insures the absence of monosulfonic acids.

**Procedure**—Add rapidly to the center of each residue 2 cc. of the phenoldisulfonic acid reagent from a pipet or buret having the tip cut off. Rapid addition of an excess of phenoldisulfonic acid prevents loss of nitrates if considerable amounts of carbonates are present.<sup>14</sup> Rotate the dish in such a way that the reagent comes in contact with all of the residue. After 10 minutes add 15 cc. of cold water to each and stir with a glass rod until the residue is in solution. Heat if necessary to dissolve. When cool, slowly add 1:2 ammonium hydroxide until slightly alkaline. Ammonium hydroxide develops a better color<sup>7</sup> and is less liable to give insoluble precipitates than potassium hydroxide.<sup>6</sup> These precipitates are probably of magnesium. They may also be prevented from forming when potassium hydroxide is used for neutralization by addition of an ammonium salt.<sup>27</sup>

Filter if necessary, transfer to a comparison tube and dilute to 50 cc. Compare with a series of standards prepared to have the same volume. Correct the result obtained for nitrite nitrogen oxidized to nitrate nitrogen, for the aliquot of the sample used, and for dilution of the aliquot.

*Balancing, Dilution and Duplication Methods.* All three of these methods are used in some cases. For the balancing or dilution method select a standard dilution of approximately the strength of the sample.

<sup>23</sup> H. D. Steenberg, *Chem. Weekblad* 14, 647-8 (1917).

A. S. Nichols, *J. Ind. Eng. Chem.* 9, 586-7 (1917).

For duplication add the colored standard to a suitable volume of water, varied according to the intensity of color of the developed sample.

*Comparison with Lovibond Glasses.*<sup>28</sup> This has been found advantageous for quick and rough estimation of nitrates by this method. Cells of 3–50 mm. were used. The reagent and procedure differed from present practice so that a new table would necessarily have to be prepared.

**Standard**—Dissolve 0.7216 gram of pure potassium nitrate in water dilute to 1 liter. Evaporate 50 cc. of this solution to dryness on the at the residue with 2 cc. of phenoldisulfonic acid and re intimate contact. Dissolve in 15 cc. of Each cc. of this solution contains 0.01 mg.

nitrate nitrogen or 0.04427 mg. of nitrate radical.

If the report is to be in terms of nitrate radical weigh 0.1631 gram of pure potassium nitrate.<sup>29</sup> Proceed with this solution as above. The resulting standard contains 0.01 mg. of nitrate radical per cc.

*Series of Standards.* As standards take 0.1, 0.3, 0.5, 0.7, 1.0, 3, 5, 10, 20, 30 and 40 cc. of the standard solution in which the color has been developed. These contain 0.002 to 0.8 mg. of nitrogen. Dilute each to about 40 cc. and add 1:2 ammonium hydroxide until faintly alkaline. Dilute to 50 cc. These standards will keep several weeks. The value of each standard in p.p.m. is 0.02 times the number of cc. of standard solution used.

The methods of the American Public Health Association<sup>30</sup> call for the use of 12 *N* potassium hydroxide solution instead of ammonium hydroxide. Add 2 cc. to each standard instead of the ammonium hydroxide. In development of color of the sample add it until a maximum color is obtained.

With that method of development of color, tripotassium nitrophenoldisulfonate,<sup>5</sup> available in a pure form, may be used as standard. For such a standard dissolve 0.295 gram of the pure salt in water and dilute to 1 liter. Use this in the same way as the above standard. If protected from light the colors will keep for years. To insure its accuracy compare the standards so prepared with standards made from the standard potassium nitrate solution.

W. Richardson and Percy

*J. Soc. Chem. Ind.* 22, 1

R<sub>1</sub>

## NITRATES BY BRUCINE

Nitrates may be estimated colorimetrically by their reaction with brucine in the presence of sulfuric acid.<sup>32</sup> A solution of brucine in chloroform is preferable to one in sulfuric acid.<sup>33</sup> A suitable sample contains 0.01 to 0.2 mg. of nitrate nitrogen. The color is intense and persists for many hours. The estimation is made by the sulfur yellow that follows the initial red color, rather than by the original color which can not be relied on. If nitrites are not to appear in the final results, 2 parts of sulfuric acid must be present for every part of water. To determine nitrites as well as nitrates, lessen the amount of sulfuric acid so that 2 parts of water will be present to 1 of sulfuric acid. If the sample solution contains much organic matter or ferrous iron oxidize by the addition of 0.1 per cent of potassium permanganate solution until it is in slight excess. If this oxidation is carried out nitrites will be oxidized to nitrates. If nitrates in the presence of nitrites are to be determined it will be necessary to determine the nitrites separately and subtract the result obtained from the total nitrate shown.

**Sample**—The samples of the previous method are suitable.

**Meat.**<sup>34</sup> Grind 10 grams with 100 cc. of water. Add sodium carbonate until alkaline and heat on a water bath for 5 hours. Add water to dilute to the original volume, cool and filter. Dilute the filtrate to 200 cc. and mix. To 25 cc. of filtrate add 12.5 cc. of a 5 per cent solution of mercuric chloride and 12.5 cc. of 2 per cent hydrochloric acid. Filter and dilute the filtrate to 500 cc. If necessary dilute further.

**Procedure**—Put 10 cc. of sample in a 50 cc. volumetric flask and 10 cc. of distilled water in a similar flask. To the standard add a suitable volume of nitrate solution containing 0.1 mg. of nitrate nitrogen per cc. A solution containing 0.7216 gram of potassium nitrate per liter is of this strength. To each add 0.2 cc. of a 5 per cent solution of brucine in chloroform and 20 cc. of concentrated sulfuric acid.

After the color has changed to yellow cool quickly and dilute each to 50 cc. with distilled water or sulfuric acid according to whether or not nitrite is to be determined. The colors may be compared any time within 24 hours.

<sup>32</sup> L. W. Winkler, *Chem.-Ztg.* 23, 454 (1899); *Ibid.* 25, 586-7 (1901).

<sup>33</sup> L. W. Haase, *Chem.-Ztg.* 50, 372 (1926).

<sup>34</sup> H. C. S. Snethlage, *Chem. Weekblad* 26, 612 (1929).

## NITRATES BY STRYCHNINE AND SULFURIC ACID

Nitrate nitrogen reacts with reduced strychnine in the presence of sulfuric acid to give a rose colored solution.<sup>35,36</sup> The reagent is sensitive to 0.01 p.p.m. of nitrate nitrogen. Except for very small amounts, a titrametric method is preferable. The method is so sensitive that many interferences due to color in the extract being examined can be eliminated by dilution.

Peptone interferes by giving a purple color with sulfuric acid. Nitrites react with the reagent before the introduction of the acid, and should be determined, if present, and the reading subtracted from that for nitrate nitrogen. Nitrites may also be corrected for by addition of an equivalent amount to the standards.<sup>37</sup> Oxidizing agents should be absent as they also give positive tests with the reagent. Chlorides do not interfere.

**Sample**—Dilute the extract, prepared according to previous methods, to 0.01 p.p.m. of nitrate nitrogen.

**Strychnine Reagent**—Keep the strychnine sulfate well protected from air. Mix 1 volume of a colorless 0.5 per cent solution of strychnine sulfate in concentrated hydrochloric acid with 1 volume of a 0.1 per cent solution of mercuric chloride in water. A 1 per cent solution of zinc chloride or a 0.002 per cent solution of lead chloride may be substituted for the mercuric chloride.

Pour 25 cc. of the mixture cautiously over 1 gram of powdered magnesium in a 300 cc. flask. The reaction is very vigorous. Several flasks may be prepared and then combined. When cool, filter or decant. The reduced strychnine should be used within a few hours.

**Procedure**—Introduce 1 cc. of reagent into each of a series of test tubes. To each sample tube add 5 cc. of the solutions to be examined, then 5 cc. of concentrated sulfuric acid. Mix each by pouring into other tubes. Treat a series of standard volumes of nitrate solution in similar test tubes in the same way as quickly as possible. The full color development takes from a few minutes to one-half hour, the latter with very dilute solutions. The color deepens slowly for a long time and may still be compared after some hours if not exposed to light. A sample must not be compared with standards developed at a time more than 5 minutes earlier or later.

<sup>35</sup> G. Deniges, *Bull. soc. chim.* [4] 9, 544 (1911).

<sup>36</sup> F. M. Scales and A. P. Harrison, *Ind. Eng. Chem.* 16, 571-2 (1924).

<sup>37</sup> I. M. Kolthoff, *Chem. Weekblad* 21, 423-4 (1924).

**Standard**—Dissolve 6.0677 grams of sodium nitrate in water and dilute to 1 liter. Each cc. corresponds to 1 mg. of nitrate nitrogen. Dilute 10 cc. to 1 liter. In this final solution 1 cc. corresponds to 0.01 mg. of nitrate nitrogen.

#### NITRATES BY DIPHENYLAMINE ON A SPOT PLATE

The blue color with diphenylamine on a spot plate may be used for a roughly quantitative estimation of nitrates, such as in field work.<sup>38</sup> The final concentration of sulfuric acid must be 70 to 90 per cent. Good results have been obtained with soil extracts and plant tissue.

**Procedure**—Prepare a series of solutions containing 1, 2, 3, 5, 7, 10, 15 and 20 p.p.m. of nitrate nitrogen. These are conveniently prepared by dilution of a solution containing 0.7216 gram of potassium nitrate per liter. This contains 100 p.p.m. of nitrate nitrogen.

Place 1 drop of each and 1 drop of the sample on a spot plate. Add 4 drops of a solution containing 0.05 gram of diphenylamine in 25 cc. of concentrated sulfuric acid to each of the drops. The solution should not be over 2 to 3 days old.

Mix well and allow to stand. The full color develops in about 2 minutes. The nitrate nitrogen in the sample may then be estimated from the series of standards. A blank with distilled water must give no blue color. Variations cannot be readily observed above 25 p.p.m. If above that concentration dilute accurately to fall within the observable range. For comparison the following table of colors may be used after some experience is gained with the colors as defined.

Nitrate Nitrogen p.p.m.	Color
1	Pale forget-me-not blue
2	Pale violet blue
3	Light violet blue
5	Dull violaceous blue
7	Phenyl blue
10	Helvetia blue
15	Hays blue
20	Cyanine blue

<sup>38</sup> M. Francis Morgan, *Science* 61, 343-4 (1930).

## NITRATES BY DIPHENYLAMINE OR DIPHENYLBENZIDINE IN SULFURIC ACID

Nitrates produce a blue color with diphenylamine in sulfuric acid, which can be used for their estimation.<sup>39-41</sup> The colored product is a salt of diphenylbenzidine, produced by oxidation.<sup>42,43</sup> Oxidation occurs in stages; diphenylamine is oxidized to diphenylbenzidine, both of which give colorless solutions, then diphenylbenzidine is oxidized to the blue imonium salt. For this reason both diphenylamine<sup>39</sup> and diphenylbenzidine<sup>40</sup> have been used to give the final product. A study of the two reagents indicates that diphenylbenzidine gives twice the color intensity produce with diphenylamine with the same amount of nitrate.<sup>46</sup> The color produced with diphenylbenzidine is a violet-blue rather than a clear blue, and is not as stable as the color produced with diphenylamine. Diphenylamine is therefore the more practical reagent, but diphenylbenzidine can be used for smaller amounts of nitrate.

The conditions for the determination must be carefully controlled, as the color increases with increasing amounts of sulfuric acid and of chlorides, as well as of nitrates.<sup>43</sup> The color decreases the lower the concentration of diphenylbenzidine. An increase in temperature increases the sensitivity of the reaction. The stability of the color depends on the proportion of diphenylbenzidine to nitrate. A large excess of diphenylbenzidine must not be present. Because of the influence of so many factors, a series of reagents is used. The optimum ratio of sulfuric acid to water is 2:3. Since the reaction depends on oxidation, other oxidizing agents must be absent. From 0.05 to 5 mg. of nitrogen per liter of solution may be determined, using diphenylamine with the higher concentrations, and diphenylbenzidine with the lower.

To estimate the nitrate content in the presence of nitrites, the nitrite as separately determined must be subtracted from the total as estimated colorimetrically. The error is within 2 per cent.<sup>43</sup>

**Sample—Soil.**<sup>46</sup> Shake 5 grams of soil, dried for 3 hours at 90°.

<sup>39</sup> E. Kopp, *Ber.* 5, 284-5 (1872).

<sup>40</sup> Edmund A. Letts and Florence W. Rea, *J. Chem. Soc.* 105, 1157-61 (1914).

<sup>41</sup> L. Smith, *Z. anal. Chem.* 56, 28-42 (1917).

<sup>42</sup> F. Kehrman and St. Micewitz, *Ber.* 45, 2645-53 (1912).

<sup>43</sup> Hans Stromberg, *Proc. Staff Meetings Mayo Clinic* 7, 254-6 (1932).

<sup>46</sup> H. Riehm, *Z. anal. Chem.* 81, 353-77, 439-47 (1930).

<sup>48</sup> May Whelan, *Proc. Staff Mayo Clinic* 4, 362 (1929); *J. Biol. Chem.* 86, 189-97 (1930).



with 50 cc. of 2 per cent potassium chloride solution for one-half hour. Filter. Use the filtrate for the determination.

**Reagents** <sup>50</sup>—A series of concentrations of reagents is necessary.

*Sulfuric Acid.* Add 2 grams of ammonium chloride to 1 liter of concentrated sulfuric acid and heat for 1 hour at 180°.

*90/100 Solution.* Add 100 mg. of diphenylamine to 900 cc. of water. Slowly add 1 liter of the prepared concentrated sulfuric acid, cooling during the addition. To 6 grams of pure ammonium chloride in a storage bottle add 1 liter of the above solution.

*80/100 Solution.* Prepare in the same way, except use 800 cc. of water.

*60/100 Solution.* Prepare in the same way, except use 50 mg. of diphenylamine, 600 cc. of water, and 3 grams of ammonium chloride in the storage bottle.

*15/100 Solution.* A. Mix 100 cc. of water with 1 liter of treated sulfuric acid.

B. To 300 cc. of water and 200 mg. of diphenylbenzidine, add 1 liter of prepared concentrated sulfuric acid. To 250 cc. of this solution add 0.2 gram of ammonium chloride.

**Procedure** <sup>50</sup>—*Preliminary.* *Reagents* 90/100 and 80/100. To 1 drop of the sample solution on a spot plate add 10 drops of the reagent. If a medium or dark blue color appears before 3 minutes have elapsed, dilute the sample at least 100-fold. If a pale blue color develops in less than 5 minutes, dilute 10 times. If a pale blue color develops in 5 to 10 minutes, dilute 1:1. If a blue color develops very rapidly, it is possible that nitrites are present. When enough nitrite is present to interfere, add to 10 cc. of sample 20 mg. of urea and 1 drop of concentrated sulfuric acid. Let stand over night.

*Final.*—*Reagent* 90/100. Place 0.5 cc. of properly diluted sample solution in a small tube. Add 5 cc. of reagent slowly so that the temperature does not rise, but do not let the addition take over 20 minutes. Stir or shake until the solution is free from cloudiness. Place 0.5 cc. each of standard solutions containing 1, 2 and 5 mg. of nitrogen per liter in similar tubes and treat in the same way. After 1 to 3 hours, compare the sample with the closest standard in a micro colorimeter.

*Reagent* 80/100. Use the same as 90/100.

*Reagent* 60/100. Use similarly, except that mixing of solution and

<sup>50</sup> K. Pfeilsticker, *Z. anal. Chem.* 89, 1-8 (1932).

reagent must take place within 10 minutes. The standards used should contain 0.1, 0.2 and 0.5 mg. of nitrogen per liter.

*Reagent 15/100.* Mix 0.5 cc. of sample solution with 1.5 cc. of sulfuric acid solution A and cool. Add 0.5 cc. of reagent B in not more than 10 minutes. Stir or shake once. Treat 0.5 cc. each of standard solutions containing 0.05, 0.1 and 0.2 mg. of nitrogen per liter in exactly the same way. Compare after 45 minutes, using a micro colorimeter.

**Standard**—Dissolve 0.722 gram of pure potassium nitrate in water and dilute to 1 liter. Add a little toluene. This solution contains 100 mg. of nitrogen per liter. When needed, dilute this stock solution to give the standards recommended.

#### NITRATES BY DIPHENYLAMINE SULFONIC ACID

Diphenylamine sulfonic acid is superior to diphenylamine as a reagent for estimation of nitrates.<sup>52</sup> Nitrites must be removed by boiling with ammonium chloride. Urea cannot be used for the purpose as it also interferes. Ferric iron also interferes.

**Sample**—To 100 cc. of solution containing nitrate, with no more than 1 mg. of nitrite, add 0.5 gram of ammonium chloride. Evaporate to 25 cc. to destroy the nitrites and dilute to 100 cc.

**Procedure**—Prepare a series of standard tubes containing 10 mg. of potassium chloride and 10 cc. of 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg. of nitrate per liter. Add about 10 grams per liter of potassium chloride to the sample and measure out 10 cc.

To each tube add 10 cc. of concentrated sulfuric acid and stir while cooling in cold water. Then add 0.1 cc. of a 0.006 *M* solution of sodium diphenylamine sulfonate and mix. Compare as soon as the standard and comparable tubes on each side of it show sufficient color. There is no exact relationship between color and nitrate concentration. Therefore, for accuracy to 5 per cent repeat with a series of standards at closer intervals and a new sample.

#### NITRATES BY PYROGALLOL

Pyrogallol sulfonic acid gives a rose color with nitrates or nitrites in

<sup>52</sup> I. M. Kolthoff and G. E. Noponen, *J. Am. Chem. Soc.* 55, 1448-53 (1933).

the presence of sulfuric acid which after 1 hour will detect 0.00005 mg. of potassium nitrate per cc.<sup>53</sup> As the amount increases the color is brownish red, olive green and finally black. Pyrogallol gives similar color reactions but the test is less sensitive.

Ferric ion gives a violet-red, iodates violet, chromates yellow and organic matter brown. Small amounts of chlorates, ferrous ion, bromides and chlorides do not interfere. Nitrites give the same colors as nitrates.

**Sample—Soil.** Shake 100 grams of soil for 1 hour with 200 cc. of distilled water. Filter and collect 80 cc. of filtrate. To this in a 100 cc. flask add 3 cc. of saturated barium hydroxide solution. Heat to boiling and let settle. Add 1 cc. of 50 per cent basic lead acetate solution. After 2 to 3 minutes add about 5 cc. of a saturated solution of sodium sulfate to remove excess lead and barium. Cool and dilute to 100 cc. Shake, filter and collect 10 cc. of filtrate. This corresponds to 4 grams of sample.

If nitrites exceed 0.1 mg. of nitrogen trioxide per kg. of earth, add 1 drop of a saturated solution of urea and 1 cc. of concentrated sulfuric acid. Nitrites disappear within 10 minutes and no nitrates are formed.

**Water.** Treat 100 cc. of water as above, omitting addition of barium hydroxide and basic lead acetate unless large amounts of ferric ion, iodide or organic matter are present.

**Reagents—Pyrogallol Sulfonic Acid.** Dissolve 5 grams of pyrogallol in 10 cc. of concentrated sulfuric acid. Heat for a few minutes at 80–90° until crystals form. When cool dissolve in water and dilute to 200 cc.

**Pyrogallol.** Dissolve 5 grams of pyrogallol in water. Add 0.2 gram of sodium bisulfate and dilute to 200 cc.

**Procedure—Low Nitrate.** To the sample in a 50 cc. porcelain dish add 0.5 cc. of pyrogallol sulfonic acid reagent. Mix and add 20 cc. of concentrated sulfuric acid. At the same time dilute different volumes of a standard nitrate solution to 10 cc. and treat each in the same way as the sample. These should cover the range of 0.00005–0.01 mg. of potassium nitrate per cc. Compare the sample after 1 hour with standards prepared at the same time.

i Umberto de Nardo, *Compt. rend.* 188, 563-5 (1929).

*High Nitrate.* If the nitrate as potassium nitrate exceeds 0.01 mg. per cc. use 5 cc. of sample. Add 5 cc. of pyrogallol reagent and mix. Add 25 cc. of concentrated sulfuric acid and mix. Compare after 1 hour with a series of standards prepared at the same time.

#### NITRATES BY REDUCTION TO AMMONIA

The micro determination of nitrates by reduction with Devarda's alloy,<sup>54</sup> 45 per cent aluminum, 50 per cent copper, 5 per cent zinc, and sodium hydroxide is satisfactory. Aluminum is also used.<sup>55</sup> Reduced iron and sulfuric acid have been stated to give more consistent results with nitrates in bismuth carbonate.<sup>56</sup> In all cases the final determination is with Nessler's reagent.

**Sodium Hydroxide Reagent**<sup>55</sup>—Dissolve 250 grams of sodium hydroxide in 1250 cc. of water. Add several strips of aluminum foil weighing about 0.5 gram each and let stand over night. Evaporate to about 1 liter. Any nitrates present as impurities are eliminated.

**Procedure**—Add 2 cc. of the sodium hydroxide solution to the sample of 100 cc. or less, according to nitrate concentration. Evaporate to about 20 cc. Transfer to a 100 cc. test tube and use wash water until the volume is about 75 cc. Close the tube with a rubber stopper and bent exit tube which leads into a smaller test tube containing about 10 cc. of 0.1 *N* sulfuric acid. This traps any ammonia evolved.

Add a strip of aluminum about  $10 \times 6 \times 0.33$  mm. and put the stopper in place. Let stand over night. Transfer the contents of both tubes to a distilling flask. Add 250 cc. of water and distil, collecting the distillate in a 200 cc. flask.

Determine the amount of ammonia in the distillate using the Nessler method described under ammonia.<sup>57</sup> Calculate back to the original nitrate content in the sample.

#### NITRIC ACID IN SULFURIC ACID BY ESTIMATION AS PICRIC ACID

Nitric acid in sulfuric acid reacts with phenol to give picric acid. The

<sup>54</sup> C. F. Flint, *J. Soc. Chem. Ind.* 46, 379-81T (1927).

<sup>55</sup> American Public Health Association, "Standard Methods for Examination of Water and Sewage," 5th ed., p. 21 (1925).

<sup>56</sup> G. J. W. Ferrey, *Quart. J. Pharm. Pharmacol.* 2, 205-16 (1929).

<sup>57</sup> See pp. 649-57.

resulting color is the basis of a method of estimation of small amounts of nitric acid in sulfuric acid.<sup>58</sup>

**Procedure**—To 20 cc. of sulfuric acid sample add 5 cc. of a mixture of 1 part of phenol, 4 parts of sulfuric acid free from oxides of nitrogen and 2 parts of water. At the same time treat similarly 20 cc. of pure sulfuric acid to which a known amount of nitric acid has been added.

Compare the yellow colors by dilution of the more deeply colored specimen with sulfuric acid known to be free from oxides of nitrogen. If the stock sulfuric acid contains oxides of nitrogen agitate with mercury in a nitrometer to remove them.<sup>59</sup>

<sup>58</sup> C. Berger, *Rev. gen. chim.* 14, 141-6 (1911).

<sup>59</sup> H. D. Steenbergen, *Chem. Weekblad* 14, 647-8 (1917).

## CHAPTER LXII

### NITRITES

#### NITRITES BY SULFANILIC ACID AND $\alpha$ -NAPHTHYLAMINE

THE red color which appears when acetic acid solutions of sulfanilic acid and  $\alpha$ -naphthylamine are acted on by nitrous acid may be used for estimation of the amount of nitrite present.<sup>1,2,3</sup> In the process the sulfanilic acid is converted by the nitrous acid into the corresponding diazo compound and the latter reacts with the  $\alpha$ -naphthylamine to form  $\alpha$ -naphthylamine-*p*-azobenzene-*p*-sulfonic acid, a red azo dye. The full color which will result does not appear for several hours but if the temperature and other conditions of the standard and sample are identical the color will appear at the same rate.

**Sample**—If the sample of water is colorless, measure 100 cc. into a cylinder. If color present cannot be removed by simple filtration, decolorize as follows: Put 200 cc. of sample in a 250 cc. glass-stoppered bottle. Add 3 cc. of alumina cream and shake. Filter after 15 minutes, discarding the first 25 cc. Take 100 cc. of filtrate as sample.

**Procedure**—Prepare a sulfanilic acid-acetic acid mixture by dissolving 3.3 grams of sulfanilic acid in 750 cc. of water by the aid of heat and adding 250 cc. of glacial acetic acid. Add 10 cc. of this to the sample. Prepare  $\alpha$ -naphthylamine acetate solution by boiling 0.5 gram of  $\alpha$ -naphthylamine in 100 cc. of water for 5 minutes, filtering through cotton, adding 250 cc. of glacial acetic acid and diluting to 1 liter. Add 10 cc. of this to the sample. Compare after 10 minutes with standards treated similarly at the same time. The color is reliable for 30 to 45 minutes. If the color developed from the sample is deeper than that of the standards repeat with another sample accurately diluted.

<sup>1</sup> L. Ilosvay de N. Ilosva, *Bull. soc. chim.* [3] 2, 388 (1889).

<sup>2</sup> F. W. Richardson and P. Hollings, *J. Soc. Chem. Ind.* 22, 616 (1903).

<sup>3</sup> A. G. Woodman and J. F. Norton, "Air, Water and Food from a Sanitary Standpoint," 4th ed., p. 80-1. John Wiley and Sons, Inc., New York, N. Y. (1914).

**Standards**—*Natural*. Dissolve 0.4926 gram of pure sodium nitrite in water and dilute to 1 liter. Dilute 100 cc. to 1 liter, and 10 cc. of the latter to 1 liter. Each cc. corresponds to 0.0001 mg. of nitrite nitrogen. For a series of standards dilute 5, 10, 15 and 20 cc. of this solution to 100 cc. with nitrite-free distilled water. These correspond to 0.005, 0.01, 0.015 and 0.02 p.p.m. of nitrite. Intermediate standards may be required. Estimation closer than 0.001 p.p.m. is unreliable.

*Artificial*. Acid fuchsin solutions may be used as approximate standards.<sup>4</sup> Dissolve 0.01 gram of fuchsin S in water and dilute to 1 liter. As standards use 3, 6, 9 and 12 cc. of this solution. Place in comparison tubes and dilute to 100 cc. with water saturated with camphor to which 10 cc. of glacial acetic acid has been added per liter. Seal the standards with paraffined corks. These correspond to 0.005, 0.01, 0.015 and 0.02 p.p.m. The standards so prepared must be checked against nitrite standards because of the many possible sources of error. Certain investigators have reported comparison with acid fuchsin unsatisfactory.<sup>5</sup> Reading the color with a Lovibond tintometer has also been used for comparison.<sup>2</sup>

#### NITRITES BY DIMETHYL- $\alpha$ -NAPHTHYLAMINE

The color produced with dimethyl- $\alpha$ -naphthylamine is superior in intensity, stability and brilliance to that with  $\alpha$ -naphthylamine.<sup>7</sup> The method is the same. The reagent is stable for at least 60 days. The effect of hydrogen sulfide is less than with  $\alpha$ -naphthylamine.

**Reagent**—Dissolve 5.25 gram of dimethyl- $\alpha$ -naphthylamine in 1 liter of 4 *N* acetic acid in 95 per cent methanol. Use as with the previous method.

#### NITRITES BY $\alpha$ -NAPHTHYLAMINE AND TARTARIC ACID

A very sensitive color reaction between  $\alpha$ -naphthylamine hydrochloride, tartaric acid and nitrite may be used for amounts of nitrite below 0.15 mg. per liter.<sup>8</sup>

**Reagent**—Dissolve 5 grams of  $\alpha$ -naphthylamine hydrochloride and

<sup>4</sup> R. Danet, *J. pharm. chim.* 7, 113-4 (1928).

<sup>7</sup> Frederick G. Germuth, *Ind. Eng. Chem., Anal. Ed.* 1, 28 (1929).

<sup>8</sup> G. Romijn, *Chem. Weekblad* 11, 115 (1914).

445 grams of tartaric acid in 50 grams of concentrated sulfuric acid. The reagent keeps indefinitely.

**Procedure**—Add 1 cc. of the reagent to 50 cc. of sample and a suitable standard. If necessary aliquot the sample to lower the nitrite content to the limits of the method. Compare by duplication, dilution or balancing.

NITRITES BY  $\alpha$ -NAPHTHYLAMINE AND  $\beta$ -NAPHTHYLAMINE-6,8-DISULFONIC ACID

Other applications of the diazo reaction have been made.<sup>8a,b</sup> That reported as most satisfactory is by coupling  $\alpha$ -naphthylamine and  $\beta$ -naphthylamine-6,8-disulfonic acid<sup>8c</sup> (Amino-G-acid), to give a violet-blue in acid solution. The reaction is so sensitive that it will detect 1 part in 750 million in a 10 cm. column. Sample and standard must be very similar in concentration or the nature of the color is altered and sample and standard cannot be compared. With more than 0.07 mg. of nitrite per cc. the color becomes red.

The reaction is not affected by nitrates, urea or uric acid, glycine, cystine, tyrosine, alanine or leucine in the concentrations normally encountered in blood. Because of its extreme sensitiveness unusual care must be taken to avoid contamination of samples and reagents.

**Sample—Blood.** Add exactly 8 cc. of freshly drawn blood to 20 cc. of a solution containing 4.5 per cent of zinc sulfate heptahydrate. The presence of sodium fluoride as an anticoagulant is immaterial. Prompt addition is necessary to prevent loss of nitrite. After mixing, this solution may stand for 24 hours without loss. Add 4 cc. of *N* sodium hydroxide solution with thorough mixing. Centrifuge. Filtration will cause about 5 per cent loss of nitrite.

**Reagents** — $\alpha$ -Naphthylamine. Boil about 0.1 gram of solid  $\alpha$ -naphthylamine with 20 cc. of water. Decant the colorless solution for use.

$\alpha$ -Naphthylamine-6,8-disulfonic acid. Add a small drop of the commercial 32.6 per cent solution of the sodium salt of Amino-G-acid to 50 cc. of water. The resulting solution is pale blue and opalescent. Add about 10 cc. of glacial acetic acid. The solution is colorless or a faint pink.

<sup>8a</sup> J. P. Griess, German Patent 3224 (1878).

<sup>8b</sup> G. I. Wallace and S. L. Neave, *J. Bact.* 14, 377-84 (1927).

<sup>8c</sup> Edward J. Stieglitz and Alice E. Palmer, *J. Pharmacol.* 51, 398-410 (1934).



**Procedure**—Mix 8 cc. of a clear solution of sample with 8 cc. of each of the reagents. At the same time treat a similar volume of a suitable standard the same way. Heat the sample and standard in a water bath at 80° to develop the maximum color. At 38° this develops in 30 minutes, at 80° in 20 minutes. No fading occurs within 18 hours.

**Standard**—*Natural*. Dissolve 0.0200 gram of sodium nitrite in water and dilute to 1 liter. Each cc. contains 0.0134 mg. of nitrite radical. For use dilute to such an extent as necessary, usually 1:100.

*Artificial*. Dissolve 0.0937 gram of potassium permanganate in water and dilute to 1 liter. The color of this is equivalent to that derived from 0.01 mg. of sodium nitrite or 0.00667 mg. of nitrite radical.

#### NITRITES BY DIMETHYLANILINE

Nitrous acid reacts with dimethylaniline to give the yellow color of *p*-nitrosodimethylaniline which may be used for colorimetric estimation.<sup>9</sup> Nitrates do not interfere. The method will detect 1.0 p.p.m.

**Procedure**—Dissolve 8 grams of dimethylaniline in 100 cc. of 1:6 hydrochloric acid. Add 0.3 cc. of this and 0.1 cc. of concentrated hydrochloric acid to 100 cc. of sample. Prepare a standard at the same time. Compare sample and standard after 15 to 30 minutes.

#### NITRITES BY METAPHENYLENEDIAMINE

Bismark brown is formed by the action of nitrous acid on metaphenylenediamine reagent.<sup>10</sup> The color is yellow to brown. The most accurate results are obtained at a pH of 2.6–2.8.<sup>11</sup> The method does not seem to be much used.

**Procedure**—Mix 2 cc. of 1:3 sulfuric acid and 1 cc. of a 5 per cent solution of metaphenylenediamine in 1:20 sulfuric acid. Add to 100 cc. of sample and compare with a series of standards.

**Standard Nitrite**—Dissolve 0.0493 gram of sodium nitrite in water and dilute to 100 cc. Mix 10 cc. with 90 cc. of concentrated sulfuric acid to give a standard of which each cc. is equivalent to 0.01 mg. of nitrogen.

<sup>9</sup> E. H. Miller, *Analyst* 37, 345 (1912).

<sup>10</sup> J. P. Griess, *Ber.* 11, 624 (1878).

<sup>11</sup> R. Uzel, *Collection Czechoslov. Chem. Communications* 5, 139-42 (1933).

## NITRITES BY ANTIPYRINE

An acetic acid solution of antipyrine gives a green color with nitrite.<sup>12</sup> The reaction will detect 50 p.p.m. Ferric salts or mineral acidity must be absent.

**Procedure**—Mix 5 cc. of sample and 5 cc. of a 1 per cent solution of antipyrine in 10 per cent acetic acid. Compare with standards similarly treated at the same time.

## NITRITES BY ZINC IODIDE AND STARCH SOLUTION

The blue color produced by nitrites in acid solution by reaction with zinc iodide-starch solution is the basis of this determination.<sup>13</sup> The method has been estimated to be 20 times as delicate as the metaphenylenediamine reaction. A color appears in 7 minutes if 0.00025 mg. of nitrite is present. The method is at best only roughly quantitative.

**Procedure**—Prepare the starch solution by boiling 5 grams of starch and 20 grams of stannous chloride in 100 cc. of water for several hours. Replace the water as it evaporates. To this solution add 2 grams of zinc iodide, dilute to 1 liter and filter. Add 4 cc. of this solution to 50 cc. of the sample acidified with 2 cc. of 1:5 sulfuric acid. The blue color develops most rapidly in the light. Compare with standards developed at the same time in the same way.

**Standard**—Prepare a standard solution of sodium nitrite by dissolving 0.4925 gram of pure sodium nitrite in water and diluting to 1 liter. Pipet out 10 cc. of this solution and dilute to 1 liter. The resulting standard solution contains 0.001 mg. of nitrite nitrogen per cc.

## NITRITES BY CONVERSION TO NITRATES

Nitrites when treated with hydrogen peroxide, potassium permanganate or other suitable oxidizing agents are oxidized to nitrates. Any of the usual methods for determination of nitrates may then be applied.

<sup>12</sup> M. C. Schuyten, *Chem.-Ztg.* 20, 722 (1896).

<sup>13</sup> E. A. Letts and F. W. Rea, *Analyst* 39, 350 (1914).

## CHAPTER LXIII

### AMMONIA

#### NITROGEN AS AMMONIA BY NESSLER'S REAGENT

IN REACTION with ammonia Nessler's reagent produces a yellow to brown color which is a very accurate indicator of the amount of ammonia present in the solution. This reaction is used for examination of many biological materials, as well as for organic nitrogen, to be discussed in detail in another volume.

**Ammonia-free Water**—Redistil 500 cc. of distilled water from a solution containing 1 gram of potassium permanganate and 1 gram of sodium carbonate. Reject the first 100 cc. portion of the distillate, after which distil about 300 cc.

**Gum Arabic Protective Colloid**<sup>1</sup>—Add 10 grams of powdered gum arabic slowly with vigorous stirring to 190 cc. of ammonia-free water and stir until the gum is completely dispersed. Transfer to a flask and add 4 grams of Permutit powder. Shake at intervals for 10 minutes and let stand to settle. After a few minutes decant the slightly turbid supernatant liquid. Test a portion to see that it gives only a faint coloration with Nessler's solution. If it gives a distinct reaction repeat the treatment with Permutit powder.

To the colloid prepared as above add about one-tenth its volume of Folin and Wu's Nessler solution to remove reducing materials, allow to stand and decant when ready for use.

By use of 2.5 cc. of this solution per 50 cc. of final solution as high as 10 mg. of nitrogen may be estimated in 50 cc. Satisfactory results have been obtained on solutions saturated with sodium sulfate.

—*Ammoniacal nitrogen in water.*<sup>2</sup> This is only the nitrogen originally present as ammonia or an ammonium salt. Set up an apparatus for distillation from a 1 liter distilling flask with a vertical block tin or

<sup>1</sup> H. M. Chiles, *J. Am. Chem. Soc.* 50, 217-21 (1928).

<sup>2</sup> R. S. Weston, *J. Am. Chem. Soc.* 22, 469-73 (1900).

aluminum condenser. Put distilled water in the flask and distil until the distillate shows no test for ammonia with Nessler's reagent. Empty the distilling flask. Place 500 cc. of sample or a smaller volume diluted to 500 cc. in the distilling flask. If acid to methyl orange add 0.5 gram of sodium carbonate.

Distil at the rate of not less than 6 nor more than 10 cc. per minute, collecting the distillate in four 50 cc. portions in Nessler tubes. Use each as sample and total the nitrogen content found in the four. If the nitrogen content is high use a 200 cc. calibrated flask as a receiver. In that case use as sample 50 cc. or an aliquot diluted to 50 cc.

*Ammoniacal nitrogen in water. Alternative method.*<sup>3</sup> If only the ammoniacal nitrogen is desired, it may be obtained by direct determination. Put 50 cc. of sample, or an aliquot diluted to 50 cc. with ammonia-free water, in a glass-stoppered graduated cylinder. Add a few drops of 10 per cent copper sulfate solution, and if the original sample contains hydrogen sulfide, also add a few drops of 10 per cent lead acetate or zinc sulfate solution. Mix well and add 1 cc. of 50 per cent sodium or potassium hydroxide solution. Mix again, let stand for the precipitate to settle, pipet an aliquot of the clear upper layer and use as sample.

*Albuminoid nitrogen in water.* This is the nitrogen liberated as ammonia by the action of alkaline potassium permanganate after expulsion of ammoniacal nitrogen.

To prepare the necessary permanganate solution, boil 1.2 liters of distilled water 10 minutes to drive off ammoniacal nitrogen. Add 16 grams of potassium permanganate and stir until dissolved. Add 800 cc. of clear 36 per cent sodium hydroxide or 50 per cent potassium hydroxide solution. Dilute to about 2500 cc. and concentrate to 2000 cc. Determine the ammonia in 50 cc. of the reagent and use as a blank.

Add 50 cc., or more, of the alkaline potassium permanganate solution to the flask from which ammoniacal nitrogen has been distilled. Continue distilling until four, or preferably five, 50 cc. portions have been collected. Use each as sample and total the results.

If desired, the distillate may be collected in a 200 or 250 cc. flask and 50 cc. or an aliquot diluted to 50 cc., used as sample.

*Organic nitrogen in water.*<sup>4,5</sup> This is all of the nitrogen present other

---

<sup>3</sup> E. B. Phelps, *Public Health Papers and Reports*, Am. Pub. Health Assoc. 29, 354 (1904); *J. Infect. Dis.* 1, 327 (1904).

<sup>4</sup> A. W. Palmer, "Report of the Univ. of Illinois," p. 60 (1903).

<sup>5</sup> E. B. Phelps, *J. Infect. Dis., Supp. I*, 255-72 (1905).

than as ammoniacal nitrogen. Evaporate 500 cc. of sample to 250 cc. to remove free ammonia. Add 5 cc. of concentrated sulfuric acid and a small piece of pumice. Mix and digest in a suitable flask until copious fumes of sulfur trioxide are given off and the liquid is colorless or very light yellow.

If necessary for complete decomposition of organic matter, add 5 grams of anhydrous sodium or potassium sulfate in order to raise the temperature of the boiling sulfuric acid. When decomposition is complete and the flask has cooled, dilute to 500 cc. with ammonia-free water and treat as for ammoniacal nitrogen.

*Sea Water.*<sup>6-8</sup> For ammonia in sea water the usual Nessler reagents have a nonsensitive region up to 0.03–0.08 mg. Beer's law does not apply below 0.02 mg. per liter with the special reagent found most satisfactory. For sea water, therefore, prepare Treadwell's form of Nessler's reagent.

*Ammoniacal nitrogen in sewage.*<sup>10</sup> Direct Nesslerization is preferable since distillation may give high results, especially after addition of sodium carbonate. To 100 cc. of sample add 1 cc. of a 10 per cent copper sulfate solution and mix. Add 1 cc. of 50 per cent sodium hydroxide solution, mix and let settle. If the supernatant liquid does not become perfectly clear repeat with a fresh sample adding the alkali first. If the sample contains hydrogen sulfide replace the copper sulfate with lead acetate and if that is ineffective, with zinc sulfate.

Dilute a suitable aliquot, which is usually 5 cc. or less, to 50 cc. with ammonia-free distilled water and use as sample.

*Ammoniacal nitrogen in sewage. Alternative method.* Use 10–100 cc. of sample diluted to 500 cc. and treat by distillation, the same as a water sample. If acid to methyl orange neutralize with 10 per cent sodium carbonate solution before distillation.

*Albuminoid nitrogen in sewage.* Determine as in water.

*Organic nitrogen in sewage by distillation and Nesslerization.* This determination must be made promptly after sampling as loss occurs progressively in unsterilized sewage. Take 100 cc. or less of sample in

---

<sup>6</sup> Henry E. Wirth and Rex J. Robinson, *Ind. Eng. Chem., Anal. Ed.* 5, 293-6 (1933).

<sup>7</sup> Cf. H. Wattenberg, *Ann. der Hydrog. Marit. Meteorol.* 59, 95 (1931); *Conseil Perm. Intern. Explor. Mer, Rapp. et Proc. Verb. Réunion*, 53, 108 (1931); *Analyst* 56, 208 (1932).

<sup>8</sup> Cf. L. H. N. Cooper, *J. Marine Biol. Assoc. United Kingdom* 18, 719 (1933).

<sup>10</sup> G. A. Johnson, "Report on Sewage Purification at Columbus, Ohio, to the Board of Public Service, p. 47 (1905).

a Kjeldahl flask and distil off the free ammonia. Add 10 cc. of concentrated sulfuric acid, 0.1 gram of copper sulfate and 5 grams of sodium or potassium sulfate. Digest over a low flame 30 minutes after it becomes colorless. When cool dilute to 250 cc. with ammonia-free water. Add a few drops of phenolphthalein solution and 50 per cent sodium hydroxide solution until alkaline. Connect with a still and distil as described under water analysis. Correct the result obtained for a blank on the reagents.

*Organic nitrogen in sewage by direct Nesslerization.* This method is less accurate than distillation but is preferable for routine work. Digest as for the distillation method. Dilute the digested liquid to 250 cc. in a calibrated flask. Pipet 50 cc. into a 100 cc. flask and neutralize with 50 per cent sodium hydroxide solution, keeping the flask cooled with water. When it shows a color to phenolphthalein dilute to volume with ammonia-free distilled water and let stand for 24 hours. Take an aliquot of the supernatant liquid as sample for Nessler determination. Correct for a blank on the reagents.

*Urine.*<sup>11</sup> This method separates the ammonia by its base exchange reaction with Permutit. In neutral or faintly acid solution with sodium salts low, the absorption is practically quantitative. In strongly alkaline solution the ammonia is liberated. A 60 to 80 mesh grade of Permutit is suitable. The method should be applicable to many other liquids.

Place 2 grams of Permutit in a 200 cc. volumetric flask. Add 5 cc. of distilled water and 2 cc. of urine. Rinse the pipet with 5 cc. of water and shake gently for 5 minutes. Rinse the sides of the flask with about 50 cc. of water from a wash bottle and decant. If there is appreciable color left rinse 2 to 3 times more. At this stage the ammonia is present in the Permutit. Add 5 cc. of water and 5 cc. of 10 per cent sodium hydroxide solution to displace the ammonia. Dilute to volume, let stand 15 minutes and pipet 50 cc. of solution as sample.

The absorbed ammonia is more difficult to remove if left in the Permutit over night. About 95 per cent of the ammonia is liberated in 3 minutes and all in 10 to 15 minutes. This may vary with the Permutit. After rinsing with water, with 2 per cent acetic acid, and again with water, the Permutit may be used again and is as efficient as before.

*Blood, urine, milk, etc.*<sup>1</sup> Take a suitable sample giving 2-5 mg. of nitrogen and digest with 1-2 cc. of a mixture of 70 cc. of concentrated sulfuric acid, 50 cc. of water, 20 cc. of 20 per cent perchloric acid, 15

<sup>11</sup> Otto Folin and

D. Bell, *J. Biol. Chem.* 29, 329-35 (1917).

grams of anhydrous sodium sulfate and 1 gram of copper sulfate.<sup>13</sup> The perchloric acid is not essential and tends to make results low with urine. Heat for at least 2 minutes after the solutions are colorless. Cool, dilute, add 5 cc. of protective colloid prepared from gum arabic, and 2 to 3 drops of Nessler's reagent as indicator. Neutralize with 10 per cent sodium hydroxide solution and dilute to 100 cc. Take 50 cc. of this as sample.

*Feed and other dry materials.*<sup>13</sup> Digest a suitable sample by the Kjeldahl method as described for sewage. Either distillation or direct Nesslerization may be used.

*Steel.*<sup>15</sup> Dissolve 3 grams of carefully washed steel turnings in 30 cc. of pure 6 N hydrochloric acid, heating if necessary. Transfer the solution to a dropping funnel connected with a flask containing 50 cc. of 60 per cent sodium hydroxide solution. The flask is connected to a condenser for distillation. Boil the solution in the flask until free from ammonia, as shown by testing the distillate with Nessler's reagent. Slowly add the solution in the dropping funnel and boil. When about 50 cc. of distillate are obtained transfer to a Nessler tube and determine nitrogen as ammonia. Continue to collect 50 cc. portions until one shows less than 0.01 mg. of nitrogen as ammonia.

*Steel. Alternative method.*<sup>16</sup> Follow the above procedure except to collect about 150 cc. of distillate in a 250 cc. flask and dilute to 250 cc. Use an aliquot as a sample. As standard, distil 25 cc. of ammonium chloride solution containing 0.01 mg. of ammonia nitrogen per cc. under the same conditions and dilute to 250 cc. Compare by duplication, running this standard into a blank until the color of the sample is matched. Each cc. of standard used is equivalent to 0.0016 mg. of ammoniacal nitrogen.

**Nessler's Reagent—Jackson's Modification.**<sup>17</sup> Dissolve 50 grams of potassium iodide in about 35 cc. of cold ammonia-free water. Add a saturated solution of mercuric chloride until a slight precipitate appears. Add 400 cc. of clear 36 per cent sodium hydroxide or 50 per cent potassium hydroxide solution. Dilute to 1 liter, let stand to sediment, and decant.

<sup>13</sup> Brainerd Mears and Robert E. Hussey, *J. Ind. Eng. Chem.* 13, 1054-6 (1921).

<sup>15</sup> W. T. Hall and R. S. Williams, "Iron, Steel and Brass," pp. 243-6. McGraw Hill Book Co., Inc., New York, N. Y. (1921).

<sup>16</sup> L. E. Barton, *J. Ind. Eng. Chem.* 6, 1012 (1914).

<sup>17</sup> D. D. Jackson, *Tech. Quart.* 13, 314-26 (1900).

This reagent, properly prepared, will give a color with 0.001 mg. of ammonia in 50 cc. of water within 5 minutes and will not produce a precipitate with a reasonable amount of ammonia within 2 hours. One cc. is normally used for a 50 cc. sample. Various other methods of preparation of the reagent have been proposed, the above being that in common use for water analysis.

*Folin-Wu Modification.* A more dilute reagent for biological use is as follows.<sup>18</sup> Put 150 grams of potassium iodide and 110 grams of iodine in a large flask. Add 100 cc. of water and 140 to 150 grams of metallic mercury. Shake vigorously until the color of iodine has nearly disappeared. Cool in water and continue shaking until the reddish iodine color has been replaced by the greenish color of the double iodide. Decant from the excess mercury and wash the latter. Dilute the solution and washings to 2 liters. Put 3500 cc. of a 10 per cent solution of sodium hydroxide, made by the dilution of a saturated solution, into a 5 liter flask. Add 750 cc. of the stock double iodide solution, and 750 cc. of distilled water. Folin uses 15 cc. of this in a total of 50 cc. containing the sample.

*Treadwell Modification.*<sup>19</sup> A special form of Nessler's reagent found best for sea water<sup>20</sup> is prepared as follows: Dissolve 115 grams of mercuric iodide and 80 grams of potassium iodide in enough water to make 500 cc. Add 500 cc. of 6 *N* sodium hydroxide solution.

*Frederick's Modification.*<sup>21</sup> Frederick sensitizes a Nessler's solution by adding a saturated solution of mercuric chloride drop by drop until a yellow turbidity is produced. The high sensitivity thus produced lasts only 2 hours. He uses 2 cc. of this solution in 50 cc. of total solution. Richmond<sup>22</sup> states that the sensitiveness of Nessler's reagent increases with age but this is questionable.

*Winkler's Modification.* Another method<sup>23</sup> of making Nessler's reagent is to dissolve 1 gram of mercuric iodide with 5 grams of potassium bromide and 2.5 grams of sodium hydroxide in 25 cc. of water. Dilute to 100 cc. with ordinary tap water. Let stand over night and use 2.5 cc. in

---

<sup>18</sup> O. Folin and H. Wu, *J. Biol. Chem.* 38, 89 (1919).

<sup>19</sup> Treadwell and Hall, "Analytical Chemistry," 6th ed., vol. I. John Wiley & Sons, Inc., New York (1927).

<sup>20</sup> Henry E. Wirth and Rex J. Robinson, *Ind. Eng. Chem., Anal. Ed.* 5, 293-6 (1933).

<sup>21</sup> R. C. Frederick, *Analyst* 50, 183 (1925).

<sup>22</sup> H. D. Richmond, *Analyst* 50, 67 (1925).

<sup>23</sup> L. W. Winkler, *Z. Unters. Nahr. Genussm.* 49, 164 (1925).



50 cc. of the sample. The use of lithium iodide and lithium hydroxide has also been suggested.<sup>24</sup>

**Procedure**—*Series of Standards.* To sample or samples add 1 cc. of Nessler's reagent, prepared according to Jackson's modification, or a corresponding volume prepared according to the other modifications. Mix well and let stand for 10 minutes. Compare with a series of standards against a white surface. If the color of any sample is deeper than the darkest standard available, mix well, take an aliquot, dilute to 50 cc. and compare.

*Balancing Method.* The method provides a procedure<sup>24a</sup> for control of the pH at which color development is carried out, which is applicable to other methods as well.

Transfer the sample to a 100 cc. volumetric flask. Take an equivalent standard in another, similar flask and dilute to about the same volume. Add 1 cc. of 0.01 *N* sodium hydroxide solution saturated with thymolphthalein. Neutralize the solutions with 0.5 *N* sodium hydroxide solution. Prepare a borate buffer by dissolving 12.404 grams of boric acid in 100 cc. of carbonate-free *N* sodium hydroxide solution and diluting to 1 liter with water. Add 20 cc. of this buffer to sample and standard. Protective colloids such as gelatine or gum arabic solution may be advantageously added at this point.<sup>24b</sup> Dilute each flask to about 97 cc. and add 2 cc. of Nessler's reagent. Dilute to volume and mix. Compare after 1 hour but not more than 2 hours. The final pH is about 12.0. The slight blue due to the indicator is the same in sample and standard.

**Standards**—*Natural.* Dissolve 0.0314 grams of ammonium chloride in water and dilute to 1 liter. This contains 0.01 mg. of ammonia nitrogen per cc. To a series of Nessler tubes add 0.0, 0.1, 0.3, 0.5, 0.7, 1.0, 1.4, 1.7, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 6.0 cc. of the ammonium chloride solution containing 0.1 mg. of nitrogen per cc. Dilute each to 50 cc. with ammonia-free water and add Nessler's reagent according to the procedure.

For more concentrated standards, in which case add protective colloid to sample and standard, dissolve 0.3140 gram of ammonium chloride

<sup>24</sup> Hans J. Fuchs, *Z. physiol. Chem.* 223, 144-6 (1934).

<sup>24a</sup> F. Alten, H. Weiland and E. Hille, *Z. Pflanzenernähr., Düngung Bodenk.* 33A, 129-33 (1934).

F. Alten and E. Hille, *Angew. Chem.* 48, 137-9 (1935).

in water and dilute to 1 liter. Each cc. of this contains 0.10 mg. of ammonia nitrogen.

*Artificial. Pla*

solution of 2.0 grams of  
hydrochloric acid  
chloride in 100 cc.

of similar acid. Dilute each to 1 liter.

As standards mix the volumes indicated in Table 7 and dilute each

Compare with natural standards and  
match the natural standards within the limit of  
accuracy of observation.

TABLE 7. PERMANENT PLATINUM-COBALT STANDARDS FOR AMMONIA  
— Nessler's REAGENT

Value in Ammonia Nitrogen	Volume of Platinum Solution	Volume of Cobalt Solution
mg.	cc.	cc.
0.000	1.2	0.0
0.001	1.8	0.0
0.002	2.8	0.0
0.004	4.7	0.1
0.007	5.9	0.2
0.010	7.7	0.5
0.014	9.9	1.1
0.017	11.4	1.7
0.020	12.7	2.2
0.025	15.0	3.3
0.030	17.3	4.5
0.035	19.0	5.7
0.040	19.7	7.1
0.045	19.9	8.7
0.050	20.0	10.4
0.060	20.0	15.0
0.070	20.0	22.0

*Chromate-Cobalt.*<sup>25</sup> Prepare an aqueous solution containing 0.8 cc. of 10 per cent potassium chromate solution and 22 cc. of 10 per cent hydrated cobalt nitrate. This corresponds to 0.01 gram of ammonia per liter. Dilute 0.5, 1, 2, 3, 5 and 10 cc. of this solution to 10 cc. They correspond to the corresponding numbers of mg. of ammonia per liter.

Danet, *J. pharm. chim.* 16, 68 (1932).

Army and Ring<sup>26</sup> were unsuccessful in attempts to match the Nessler standards for water of the American Public Health Service with their permanent standards.

#### AMMONIA NEPHELOMETRICALLY BY A MODIFIED NESSLER'S REAGENT

Nessler's reagent tends to give a cloud if the concentration of ammonia is relatively high. The color developed is also affected by dissolved salts. A modified form of Nessler's reagent has therefore been developed for nephelometric use.<sup>27</sup> This reagent is sodium mercuric chloride instead of the potassium mercuric iodide used as Nessler's reagent. This is more stable and the complex does not have the appreciable ammonia vapor tension of the corresponding ammonia-potassium mercuric iodide complex.

Salts do not affect the formation of the colloidal precipitate. As a matter of good practice the amount in the sample is preferably duplicated in the standard. The method will detect 1 part of ammonia in 160 million parts of solution.

**Sample**—Many of the previous methods of preparation may be used. Kjeldahl digestion requires some modification as follows. Weigh 0.1 gram or more of sample into a Kjeldahl flask. Add 20 cc. of concentrated sulfuric acid, 10 grams of potassium sulfate and about 0.5 gram of mercuric oxide. Copper must not be used because of the color it would impart to the final solution. Digest as usual in the Kjeldahl method. Dilute the digested sample to 500 cc. Pipet 5 cc. and add 5 cc. of *N* sodium hydroxide solution. Continue this addition until neutral to litmus paper. Dilute to 200 cc. and take the aliquot for development of color from this dilution.

**Reagent**—Add 80 grams of sodium chloride to 130 cc. of water, free from ammonia.<sup>28</sup> Add 100 cc. of a cold saturated solution of mercuric chloride and shake. When solution of the salt is complete add 70 cc. of a 1 per cent solution of lithium carbonate. This is practically a saturated solution. Make this addition slowly with shaking so that no mercuric oxide forms on the sides of the flask. Some cloud is usually present due to ammonia in the reagents.

Shake the reagent with 3 to 5 grams of finely powdered talc and

<sup>26</sup> H. V. Army and C. H. Ring, *J. Ind. Chem.* 8, 309-17 (1916).

<sup>27</sup> Sara S. Graves, *J. Am. Chem. Soc.* 37, 1171 (1915).

<sup>28</sup> See p. 649.

filter. It may be used at once and will keep indefinitely if properly protected.

**Procedure**—To one comparison tube add 10 cc. of sample. To another add 10 cc. of the ammonium sulfate standard containing excess of potassium sulfate. To each add 15 cc. of a 0.003 per cent starch solution prepared by boiling 1 gram in 100 cc. of ammonia-free water and diluting 3 cc. to 1 liter. To each add 5 cc. of reagent. Compare nephelometrically.

**Standard**—Add 10 cc. of 7.5 per cent potassium sulfate solution to 10 cc. of an ammonium sulfate solution containing 94.3 mg. per liter. Dilute to 100 cc. Each cc. is equivalent to 0.002 mg. of ammonium sulfate.

#### AMMONIA BY PHENOL AND SODIUM HYPOCHLORITE

The reaction of ammonia with phenol and sodium hypochlorite is a method of estimation more rapid than the procedure with Nessler's reagent but not one admitting of as great accuracy.<sup>29,30</sup> For careful work its accuracy is not to be depended on. The time for a complete determination should not exceed 4 minutes. The method is applicable to all cases where ammonia is to be determined. Calcium does not affect the results but the presence of a considerable amount of free acid interferes. The method will detect 0.0001 mg. of ammonia in a 5 cc. sample.

**Sample**—Prepare as for the previous methods.<sup>31</sup>

**Procedure**—To 5 cc. of the sample solution add 1 cc. of a 5 per cent solution of sodium hypochlorite and 1 cc. of a 4 per cent solution of phenol. Dilute to 10 cc. Heat in boiling water for 2 minutes to develop the full intensity of color. Compare with a series of standards or by balancing or dilution.

**Balancing Method.** Treat 5 cc. of a suitable standard in the way as the sample. The concentration of standard<sup>32</sup> to be used varies according to the concentration of the sample.

**Series of Standards.** Prepare further dilutions of the standard am-

<sup>29</sup> P. Thomas, *Bull. soc. chim.* 11, 796 (1912).

<sup>30</sup> G. E. Foxwell, *Gas World (Coking Section)* 64, 10

<sup>31</sup> See pp. 649-53.

monia solution containing 0.01 mg. per cc. in 1:5 and 1:25 dilutions. Prepare standards from these and if desired from the undiluted standard,<sup>32</sup> treating each exactly the same as the sample.

#### AMMONIA BY SILVER NITRATE AND TANNIN

A fresh mixture of silver nitrate and tannin gives a color due to formation of free silver which is stated to be more sensitive than Nessler's reagent.<sup>33</sup>

**Procedure**—To 1 cc. of sample add 2 drops of 5 per cent tannin solution and 1 drop of 20 per cent silver nitrate solution. A color appears at once and may be compared with a series of standards after 1 minute.

---

<sup>32</sup> See p. 655.

<sup>33</sup> Konstantin C. Makris, *Z. anal. Chem.* 81, 212-4 (1930); *Ibid.* 84, 241-2 (1931).

## CHAPTER LXIV

### HYDROGEN-ION—GENERAL

THE titration of an acid is a measure of the total acidity present without expressing its activity. The concentration of hydrogen-ion,  $C_H$ , in a solution is a function of concentration of acid and degree of ionization. These definitions apply equally readily to bases, and, by suitable consideration of the equilibria involved, to hydrolysis accompanied by ionization of a product of hydrolysis to give hydrogen- or hydroxyl-ion.

The values of  $C_H$  may vary so greatly that charting on a linear scale is not practical. The values of  $C_H$  are therefore commonly expressed as the

pH value which is the logarithmic value,  $\log_{10} \frac{1}{C_H}$ .

**Theory**<sup>1</sup>—A simple acid, HA, in a polar solvent dissociates into  $H^+$  and  $A^-$ . If other solutes are absent and the ionization of the solvent is negligible,

$$(1) \quad H^+ = A^-.$$

At a concentration  $C$

$$(2) \quad C = HA + A^- = HA + H^+.$$

A familiar form of the law of mass action is

$$(3) \quad \frac{\times}{HA}$$

Since  $H^+ = A^-$ , this may be expressed

$$(4) \quad K_a = \frac{H^{+2}}{C - H^+}.$$

The symbol  $\alpha$  is conventionally used as a measure of the degree of dissociation and in this simple case  $\alpha = \frac{H^+}{C} = \frac{A^-}{C}$ .

---

<sup>1</sup> For a more complete discussion of the theory see W. Mansfield Clark, "The Determination of Hydrogen Ions," 3rd ed. Williams and Wilkins Co., Baltimore, Md. (1928).

In mixtures (3) holds within limits. Variation of concentration may alter it. Within the limits in which (3) hold it may be restated

(5)

From this it follows that

(6)

To express this in pH as defined, the logarithm of the reciprocal of each side of (6) gives

$$(7) \quad pH = -\log \frac{1}{\alpha} = \log \frac{1}{K_a} + \log \frac{\alpha}{1 - \alpha}.$$

Similarly

$$(8) \quad pK_a = \log \frac{1}{K_a}$$

From (7)

$$(9) \quad pH = pK_a + \log \frac{\alpha}{1 - \alpha}, \text{ or } pK = pH - \log \frac{\alpha}{1 - \alpha},$$

or if the ionization of the salt present is practically 100 per cent and the acid is but little ionized

$$(10) \quad pH = pK_a + \log \frac{\text{salt}}{\text{acid}}.$$

For convenience it is customary to consider bases only as the negative form of acids, the values of  $C_{OH}$ ,  $pOH$  and  $pK_b$  being of use only in special cases. For convenience in referring to indicators, the pH at which the amounts of acid and alkaline forms are equal is called the  $pK$  value.

An indicator is a compound undergoing complex molecular rearrangement with change of pH. One form may be colorless or the two forms may differ in color. The reaction in one direction is normally formation of a salt. Therefore inherently it is a chemical reaction of the indicator with hydrogen—or hydroxyl-ion as one of the reacting substances.

**Absorption Curves**—The absorption curve of the indicator is fundamental in colorimetric pH work. The variation of this with pH is well illustrated in Figure 96 which shows the curve for bromothymol blue, a

dichromatic indicator.<sup>2</sup> The values plotted are  $-\log T$  as determined at different wave lengths and different pH values. All values for a given curve are at the pH value indicated. The change in absorption which takes place with change of pH is ascribed to change of dissociation of the indicator. This is based on the justifiable assumption of distinctly different absorption by the ion and molecule of the indicator.

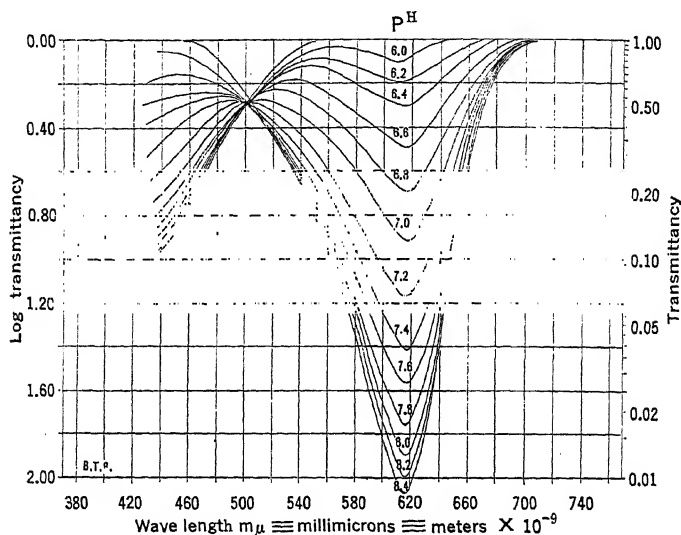


FIG. 96

Transmittancy Curve for Bromothymol Blue

The absorption of energy by the ions may be expressed by

$$(11) \quad \frac{I_2}{I} = 10^{-l\alpha K_i}$$

In this  $I_1$  is the intensity of the light entering the solution,  $I_2$  that leaving,  $l$  is the depth of the solution,  $c$  is the concentration of indicator present,  $\alpha$  is the degree of dissociation and  $K_i$  is an index of transmission of light by the ions in terms of moles per liter.

Similarly the absorption by the molecules may be expressed by

$$(12) \quad \frac{I_3}{I_1}$$

<sup>2</sup> W. R. Brode, *J. Am. Chem. Soc.* 46, 581 (1924).



in which  $I_3$  is the intensity of energy leaving the solution and  $K_m$  is the index of transmission by molecules, in moles per liter.

The value of the total transmittance is  $\frac{I_2}{I_1} + \frac{I_3}{I_1}$ .

Therefore by substitution in the general form of the equation for Beer's law,

$$(13) \quad -\log T =$$

there results

$$(14) \quad -\log T =$$

When  $\alpha$  is 1 the value for  $K_m$  disappears and the simple form is obtained,

$$(15) \quad -\log T = lcK_i.$$

Similarly, if instead of a pH value where dissociation is complete, one is assumed without dissociation the simplified form due to disappearance of  $K_i$  would be

$$(16) \quad -\log T = lcK_m.$$

At an intermediate point the curve must pass through a stage at which  $\alpha$  is 0.5. Equation (4) then becomes

$$(17) \quad -\log T = lcK_i = lcK_m.$$

The point at which this occurs is that through which all the curves for that indicator must pass. This isobestic point<sup>3</sup> is shown at about  $\lambda = 510$  in Figure 96 for bromothymol blue. The occurrence of such a point is adequate evidence that the change occurring with change in pH is no more complex than the attainment of an equilibrium between two components. When such a point cannot be determined by spectrophotometric absorption curves of the indicators it is sufficient evidence that such a simple relationship does not exist for that indicator.

Knowing that such a relationship exists, it follows that when ionization of the indicator is approximately complete, (15) becomes approximately

$$(18) \quad -\log T =$$

Having  $-\log T_a$ , the value when the absorption is at a maximum, the pH value of a well buffered solution at a value where  $\alpha$  is 0.1–0.9, and  $-\log$

<sup>3</sup> E. B. R. Prideaux, *Chem. and Ind.* 45, 664, 678 (1926).

$T_d$  for the solution at the pH value determined, it follows that with the depth of layer,  $l$ , and concentration constant

If the concentration or depth of layer is altered, the more complex form

$$(20) \quad \alpha = \frac{1}{\log T_d l_d} \quad \text{is obtained.}$$

Since only one wave length is used  $K_1$  cancels from (20) and  $c_a$  and  $c$

$$(21) \quad \alpha = \frac{1}{(\log T_d) \lambda_i}$$

$$(22) \quad (\log T_d) \lambda_m$$

Therefore

$$(23) \quad \log \frac{R_i}{R}$$

corres

<sup>4</sup> W. C. Holmes, *J. Am. Chem. Soc.* **46**, 627 (1924).

pression  $C_H \times C_{OH} = 0.0_{13}1$  defines the concentration of either when the other is known.

The pH scale is by definition the negative exponent of 10 which will give the concentration of hydrogen-ion. The concentration of hydroxyl-ion is simultaneously defined. For a value of  $C_H = 0.0_61$  the corresponding figure is  $10^{-7.0}$ . Therefore this is expressed as pH 7.0. For  $C_H = 0.0_{11}1$  this is expressed as pH 12.0. Since the acidity is less than at neutrality the concentration of hydroxyl-ion is high and pH 12.0 is a relatively high alkalinity.

TABLE 8. INTERRELATION OF pH,  $C_H$  AND  $C_{OH}$ 

pH	$C_H$ Moles Per Liter	$C_{OH}$ Moles Per Liter	Moles $H^+$ Per Liter to Change from Previous pH	Moles $OH^-$ Per Liter to Change from Previous pH
1	$10^{-1} = 0.1$	$10^{-13} = 0.0_{12}1$		
2	$10^{-2} = 0.01$	$10^{-12} = 0.0_{11}1$	0.99	+0.0 <sub>12</sub> 99
3	$10^{-3} = 0.0_21$	$10^{-11} = 0.0_{10}1$	0.099	+0.0 <sub>11</sub> 99
4	$10^{-4} = 0.0_31$	$10^{-10} = 0.0_91$	0.0 <sub>2</sub> 99	+0.0 <sub>10</sub> 99
5	$10^{-5} = 0.0_41$	$10^{-9} = 0.0_81$	0.0 <sub>3</sub> 99	+0.0 <sub>9</sub> 99
6	$10^{-6} = 0.0_51$	$10^{-8} = 0.0_71$	0.0 <sub>4</sub> 99	+0.0 <sub>8</sub> 99
7	$10^{-7} = 0.0_61$	$10^{-7} = 0.0_61$	0.0 <sub>5</sub> 99	+0.0 <sub>7</sub> 99
8	$10^{-8} = 0.0_71$	$10^{-6} = 0.0_51$	0.0 <sub>6</sub> 99	+0.0 <sub>6</sub> 99
9	$10^{-9} = 0.0_81$	$10^{-5} = 0.0_41$	0.0 <sub>7</sub> 99	+0.0 <sub>5</sub> 99
10	$10^{-10} = 0.0_91$	$10^{-4} = 0.0_31$	0.0 <sub>8</sub> 99	+0.0 <sub>4</sub> 99
11	$10^{-11} = 0.0_{10}1$	$10^{-3} = 0.0_21$	0.0 <sub>9</sub> 99	+0.0 <sub>3</sub> 99
12	$10^{-12} = 0.0_{11}1$	$10^{-2} = 0.01$	0.0 <sub>10</sub> 99	+0.0 <sub>2</sub> 99
13	$10^{-13} = 0.0_{12}1$	$10^{-1} = 0.1$	0.0 <sub>11</sub> 99	+0.099

There remains only to indicate more specifically the relationship between pH and actual concentration of hydrogen- or hydroxyl-ion.  $C_H$  is a linear relationship, each unit of change being the same as the previous. pH is a logarithmic relationship, each unit of change being ten times the previous one. This is more clearly shown by the accompanying Table 8. From this it will be noted that addition of 0.0<sub>5</sub>99 mole of hydroxyl-ion will change the pH value from 8.0 to 9.0. Addition of another 0.0<sub>5</sub>99 mole of hydroxyl ion will not change the pH value from 9.0 to 10.0. Rather it will require 10 times that amount or 0.0<sub>4</sub>99 moles to change to pH 10.0.

Table 9 gives the approximate pH of 0.1 *N* solutions of some common acids, bases and salts as illustrations of the variation of pH among solutions of like concentration.

TABLE 9. pH VALUES FOR 0.1 *N* SOLUTIONS, AT 20°, ROUNDED TO THE FIRST DECIMAL

<i>Acidic</i>	<i>pH</i>
Hydrochloric acid .....	1.0
Sulfuric acid .....	1.2
Phosphoric acid .....	1.5
Acetic acid .....	2.9
Alum .....	3.2
Carbonic acid .....	3.8
Boric acid .....	5.2
<i>Basic</i>	
Sodium bicarbonate .....	8.4
Borax .....	9.2
Ammonia .....	11.3
Sodium carbonate .....	11.6
Trisodium phosphate .....	12.0
Sodium metasilicate .....	12.2
Sodium hydroxide .....	13.0

The uses of pH determination colorimetrically are so varied that a complete listing of references, even without discussion, is not feasible within the space available. Therefore those discussed are only a selected few, illustrative of the types of application.

**Colorimetric Principles**—As the hydrogen-ion concentration of a solution containing a colorimetric pH indicator is changed, the color as well as the intensity of color is usually changed. There are some exceptions, the so-called monochromatic pH indicators.

Two types of methods are applicable, the series of standards and balancing methods.

**Principle of Buffer Solutions**—At fixed points for standards it is necessary to prepare mixtures of related compounds such that on dilution with water there is to all practical purposes no change in the pH of the solution. Probably the most familiar of these to chemists in general is an acetic acid-sodium acetate mixture.

There are numerous series of such standard buffers as outlined by Sorensen, Walpole, Clark and Lubs, and others. The quoted pH of the buffer solution depends primarily on determination by electrometric methods.

Rather than duplicate in a buffer solution the actual pH found in

the sample two other methods are possible according to the Ostwald theory. The first is to have definite volumes of acid and alkaline forms of the indicator, the two varying in concentration, the sum of the colors to be compared with the sample. The second is to vary the depths of acid and alkaline forms of the indicator in the same concentration as in the sample, the total depth of standard always being the same as that of the sample. The first is the method of Gillespie, the second is used in certain instrumental methods of measurement.

**Sources of Error**—In addition to the usual sources of error, in colorimetric determinations there are several which are particularly applicable to pH determinations.<sup>5</sup>

*Dilution of Sample.* Some samples can be diluted with an equal volume of distilled water, or even several volumes of distilled water without affecting the pH. This is because they are heavily buffered. Similar dilution of slightly buffered samples will cause relatively large errors.

*Filtration of Sample.*<sup>5a</sup> Filtration of neutral distilled water through paper does not appreciably alter the pH. Filtration of a *N* potassium chloride solution, even though the filter is first washed with distilled water, causes substantial error. If the filter is washed 4 times with *N* potassium chloride solution and the first few cc. of filtrate from a *N* potassium chloride extract are discarded the errors are largely avoided.

*Acid Contamination.* If ordinary paraffin is used for coating a bottle it gives a distinctly acid reaction and will affect an indicator or the buffer to be used. An alkaline buffer, or indicator adjusted on the alkaline side, will react with carbon dioxide of the air. This is serious above pH 8.0. If a tube is closed with the finger to shake it, distinctly acid contamination may occur.

*Alkaline Contamination.* Buffers or indicators stored in glass bottles are apt to react with the alkalinity of the glass. Water boiled even in a Pyrex flask shows an alkaline reaction on standing for 12–24 hours.

*Salt Error.* Two solutions having different concentrations of salts and the same concentration of indicator do not always show the same pH value. Therefore, strictly speaking, sample and buffers used for comparison should contain the same concentration of the same salts,<sup>5b</sup> an obvious impossibility and a condition but rarely approached in practical

<sup>5</sup> J. W. Schlegel and A. H. Stueber, *Ind. Eng. Chem.* 19, 631-3 (1927).

<sup>5a</sup> Viktor K. Neugebauer, *Bull. soc. chim. ray. Yougoslav.* 5, 73-81 (1934).

<sup>5b</sup> Martin Kilpatrick, *Chem. Rev.* 16, 57-66 (1935).

use. The cause of this change of colorimetric pH by the presence of salts is unknown.<sup>6</sup> Neither the acid nor alkaline colors of bromophenol blue, bromocresol green, bromothymol blue, phenol red, cresol red or thymol blue are affected. The acid colors of methyl orange, tropaeolin 00 and methyl red and the alkaline colors of various nitrophenols and salicyl yellow are intensified by the presence of salts.

The same types of indicators do not always behave alike. Methyl red and methyl orange show very small salt errors under different conditions, probably due to their amphoteric character. Amphoteric indicators may therefore be best. There are grave discrepancies for phenolphthalein, *o*-cresolphthalein, thymol blue and phenol red.<sup>7</sup>

The theory of Debye and Hückel may be used as a quantitative expression of the relation between ionic strength and pK values on the assumption that all the salts are completely dissociated, and that the combined effect of their ions on the logarithm of an activity coefficient is proportional, as a first approximation, to the square root of the ionic strength.<sup>8</sup>

Since the electrometric method is made the standard the effect of salt is thrown on the indicator. It is well known that salt affects both the electrometric pH and the colorimetric value. Salt errors may be positive or negative. In sea water<sup>9</sup> a 3.5 per cent salt content gives a value of pH 8.43 with phenolphthalein against a standard borate buffer when the electrometric value is 8.22. The error varies with the indicator.

TABLE 10. SALT ERRORS WITH CLARK AND LUBS INDICATORS.

INDICATOR	CORRECTION		
	1 molar	2 molar	3 molar
Thymol blue (alkaline range)	—0.22	—0.29	—0.34
Cresol red.....	—0.28	—0.32	—0.37
Phenol red.....	—0.21	—0.26	—0.29
Bromothymol blue.....	—0.19	—0.27	—0.29
Bromocresol purple.....	—0.26	—0.33	—0.31
Bromocresol green.....	—0.26	—0.31	—0.29
Bromophenol blue.....	—0.28	—0.37	—0.43
Thymol blue (acid range)....	—0.10	—0.13	—0.12
Methyl red.....	—0.04	—0.01	+0.12

<sup>6</sup> I. M. Kolthoff, *J. Phys. Chem.* 32, 1820-33 (1928).

<sup>7</sup> J. W. McBain, M. E. Laing and O. E. Clark, *J. Gen. Physiol.* 12, 695-710 (1929).

<sup>8</sup> J. Sendroy, Jr. and A. B. Hastings, *J. Biol. Chem.* 82, 201 (1929).

<sup>9</sup> S. P. L. Sorensen and S. Palitzsch, *Compt. rend. Lab. Carlsberg* 9, 8 (1910).

Numerous sets of data have been published<sup>10-13</sup> but are not reproduced here. One set<sup>14</sup> as related to the Clark and Lubs indicators is shown as Table 10.

*Protein Error.* The effect of protein on colorimetric pH values is similar to the salt error. Blood serum is a good example. Colorimetric pH on diluted serum differs by varying amounts at 20° or 38° from that of the undiluted serum.<sup>15</sup> The results are consistently 0.08 pH higher than those by the hydrogen electrode and 0.14 higher than by the quinhydrone electrode.<sup>16</sup> Other investigators have found variable differences between the colorimetric and hydrogen electrode methods and do not recommend the colorimetric method for accurate comparisons, although suitable for certain statistical studies when average correction values<sup>17</sup> are used.

The relation between colorimetric and hydrogen electrode determinations on blood plasma after hemorrhage in dogs varies considerably.<sup>18</sup> For normal sera it varies from 0.35 to 0.42, with variations in an individual animal from 0.24 to 0.41.<sup>19</sup>

It is probable that this type of error is incorrectly named from one manifestation, since the same type of error occurs with some colorimetric indicators in soap solutions. In that case it is accentuated by increasing pH with the accompanying increase in colloidal micellar soap. A more general name for the effect would be colloidal error as signifying adsorption and other phenomena occurring in colloidal solutions.

*Indicator Error.* This anomalous designation refers to the fact that the indicator is itself a factor in the pH of the solution. In producing a definitely colored form of the indicator the pH of the solution has been altered. In heavily buffered solutions this is of no importance; in unbuffered solutions it introduces serious error unless the pH of the

<sup>10</sup> E. B. R. Prideaux, "The Theory and Use of Indicators," Constable & Co., Ltd., London (1917).

<sup>11</sup> I. M. Kolthoff, *Rec. trav. chim.* 42, 186 (1923).

<sup>12</sup> W. D. Ramage and R. C. Miller, *J. Am. Chem. Soc.* 47, 1230 (1925).

<sup>13</sup> B. Cohen, *U. S. Pub. Health Repts.* 41, 3051 (1927).

<sup>13a</sup> A. Thiel and G. Coch, *Z. anorg. allgem. Chem.* 217, 353-75 (1934).

<sup>14</sup> L. B. Parsons and W. F. Douglas, *J. Bact.* 12, 262 (1926).

<sup>15</sup> J. H. Austin, W. C. Stadie and H. W. Robinson, *J. Biol. Chem.* 66, 505-19 (1925).

<sup>16</sup> G. E. Cullen and Imogene P. Earle, *J. Biol. Chem.* 76, 583-90 (1928).

<sup>17</sup> Charles G. Johnston, *J. Biol. Chem.* 79, 297-307 (1928).

<sup>18</sup> Mary A. Bennett, *J. Biol. Chem.* 69, 697-702 (1926).

<sup>19</sup> G. E. Cullen, *J. Biol. Chem.* 52, 501 (1922).

indicator is adjusted to match that of the sample before adding it. Such adjusted indicators known as "isohydric" indicators are in use.<sup>19a</sup>

*Temperature.* It is essential that the temperature of sample and standards be very nearly the same.

*Miscellaneous.* The indicator solution will fade if exposed to ultra-violet radiation.<sup>20</sup> Such exposure has no effect on the crystals. Differences in the method of preparation of the indicator solutions may affect their color. Indicators from different sources have been found to show important variations. These last two do not have any bearing provided the same indicator solution is used for sample and standards.

Among other errors that must be mentioned are the specific effects of certain buffers on indicators, slow change in color, and precipitation or change in size of colloidal particles.

<sup>19a</sup> See p. 694.

<sup>20</sup> M. G. Mellon and G. W. Ferner, *J. Phys. Chem.* 35, 1025-43 (1931).



## CHAPTER LXV

### HYDROGEN-ION BUFFERS AND INDICATORS

**Simple Standard Buffer**—A buffer for pH 7.0 can be prepared very readily by mixing equivalent solutions of ammonium hydroxide and acetic acid.<sup>1</sup> The acid and base have the same dissociation constant so that the buffer gives the neutral point. Bubbling air through the buffer for 5 minutes reduces the pH only 0.05 unit.

**Clark and Lubs Buffer Solutions**—The series from pH 2.2 to 10.0 is satisfactorily covered by a series of buffers composed of potassium chloride, monopotassium phosphate, acid potassium phthalate, boric acid with potassium chloride, sodium hydroxide and hydrochloric acid. The solutions required are as follows:<sup>2</sup>

*0.2 M Potassium Chloride.* Unless the salt is of unquestionable purity recrystallize 2 to 3 times. Dry at 120° for 2 days. Dissolve 14.912 grams in warm water and when cool dilute to exactly 1 liter.

*0.2 M Acid Potassium Phthalate.* For this, acid potassium phthalate is prepared as follows.<sup>3</sup> Dissolve 60 grams of potassium hydroxide of the highest purity in about 400 cc. of water. Add 50 grams of commercial resublimed phthalic anhydride. Commercial phthalic anhydride not resublimed may be seriously contaminated with benzoic acid, naphthols and quinones.<sup>4</sup> As much as 10 recrystallizations may be required if a good commercial grade is not used. After the phthalic anhydride has dissolved and cooled, test a portion with phenolphthalein. If still alkaline add roughly weighed portions of phthalic anhydride until a diluted portion shows only a faint pink with phenolphthalein. If acid, add more potassium hydroxide solution to faint alkalinity.

When the solution has been adjusted to faint alkalinity add 60 grams more of phthalic anhydride, plus an amount equal to that added to

<sup>1</sup> Roger J. Williams and Carl M. Lyman, *J. Am. Chem. Soc.* 54, 1911-2 (1932).

<sup>2</sup> W. M. Clark and H. A. Lubs, *J. Biol. Chem.* 25, 479 (1916); *J. Bact.* 2, 1, 109, 191 (1917).

<sup>3</sup> F. D. Dodge, *J. Ind. Eng. Chem.* 7, 29 (1915); *J. Am. Chem. Soc.* 42, 1655 (1920).

<sup>4</sup> C. Conover and H. D. Gibbs, *J. Ind. Eng. Chem.* 14, 120 (1922).

remove excess alkalinity, and heat until solution is complete. Filter while hot into a crystallizing dish. Cover and place where it will cool slowly. Filter with suction and recrystallize twice from distilled water. Do not allow the temperature to go below  $20^{\circ}$  at any time or a more acid salt,  $2\text{KHC}_8\text{H}_4\text{O}_4 \cdot \text{H}_2\text{C}_8\text{H}_4\text{O}_4$ , will deposit.<sup>3</sup> This is in prismatic needles, easily distinguished from the 6-sided, orthorhombic plates of the usual acid salt. Dry at  $110^{\circ}$  to constant weight.

Dissolve 40.836 grams in water and dilute to 1 liter. If the purity of the salt is subject to any question titrate a portion of this solution.

*0.2 M Monopotassium Phosphate.* Recrystallize the best available grade of monopotassium phosphate,  $\text{KH}_2\text{PO}_4$ , 3 times from distilled water. A suitable method of preparation of the acid of the highest quality has been described.<sup>6</sup> This can then be converted into the pure salt.

Dry to constant weight at  $110^{\circ}$ . Dissolve 27.312 grams in water and dilute to 1 liter. Test the solution to insure that it gives a distinct red with methyl red and a distinct blue with bromophenol blue.

If a sediment is deposited it is largely colloidal iron and aluminum compounds, free from silica.<sup>7</sup> The use of hard and soft glass, paraffin or silver has apparently no relation to this. As a satisfactory method of further purification, if necessary, prepare an approximately 0.2 *M* solution. Seal in a flask to prevent excessive loss of water and heat at  $75\text{--}85^{\circ}$  for 24 hours. Filter through very fine paper and evaporate the filtrate to crystallization. Crystallization of this partially concentrated solution may be facilitated by addition of an equal volume of 95 per cent alcohol.

*0.2 M Boric Acid—0.2 M Potassium Chloride.* Recrystallize boric acid several times from distilled water. Air-dry in thin layers between filter papers. It begins to lose water of constitution above  $50^{\circ}$ . Insure constant weight by drying weighed samples in thin layers over calcium chloride in a desiccator.

The quality of the potassium chloride was previously discussed. Potassium chloride is added to the boric acid solution to bring the salt concentration in the boric acid solution to a value comparable with that of the phosphate mixtures. Colorimetric checks can then be obtained when the series overlap. Dissolve 12.4048 grams of boric acid and 14.912 grams of potassium chloride in water and dilute to 1 liter.

*0.2 M Sodium Hydroxide.* Absence of any substantial amount of

<sup>6</sup> W. H. Ross, R. M. Jones and C. B. Durgin, *Ind. Eng. Chem.* 17, 1081 (1925).

<sup>7</sup> R. Holcomb and R. R. McKibbin, *J. Am. Chem. Soc.* 50, 1695-6 (1928).

carbonate in the final solution is essential. Dissolve 100 grams of the best quality sodium hydroxide in 100 cc. of distilled water in a Pyrex flask. Cover the mouth of the flask and allow to stand over night for the carbonate to settle.

If the separation is complete by sedimentation it need not be filtered. Otherwise treat a hardened filter paper with warm 1:1 sodium hydroxide solution. After a few minutes decant and wash the paper once with absolute alcohol, twice with 50 per cent alcohol and several times with distilled water. Place in a Büchner funnel and apply gentle suction until nearly dry, but not dry enough to curl. Pour the concentrated alkali on the middle of the paper and spread carefully with a glass rod, using gentle suction to make the paper adhere properly to the funnel. Filter and dilute after rough calculation to about 1.2 *M*. Standardize this exactly against a standard acid, using not less than 10 cc. as sample. From the average of not less than three standardizations agreeing within 0.001 *M* calculate the dilution to an exactly 0.5 *M* solution. Carry out the dilution with the least possible exposure and transfer to a heavily paraffined bottle to which are attached a 50 cc. buret and a soda-lime guard tube as shown in Figure 97.<sup>8</sup>

Weigh<sup>9</sup> out several portions of acid potassium phthalate. Pass a stream of carbon dioxide-free air through the solution, add phenol-phthalein and titrate with the alkali to a faint pink. If the solution varies from 0.5 *M* use a factor rather than adjust to exactly 0.5 *M*.

**0.2 *M* Hydrochloric Acid.** Dilute pure hydrochloric acid to approximately 0.2 *M* and standardize with the 0.2 *M* sodium hydroxide solution.

**Kolthoff and Vleeschhouwer Buffer Solutions**—For high pH values, up to 12.0, other buffer solutions can be used.<sup>10</sup>

**0.05 *M*. Borax.**<sup>11</sup> Recrystallize the commercial material twice from water. Dry over hydrated sodium bromide,  $\text{NaBr} \cdot 2\text{H}_2\text{O}$ , to constant weight. Dissolve 19.10 grams in water and dilute to 1 liter.

**0.05 *M* Sodium Carbonate.** Dry the best quality of sodium carbonate

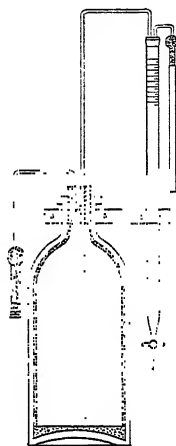


FIG. 97

Paraffined Bottle  
With Buret and  
Soda-Lime Guard  
Tube for  
Standard Alkali  
Solution

<sup>8</sup> W. Mansfield Clark, "Determination of Hydrogen Ions," 3rd ed., p. 196. Williams and Wilkins Co., Baltimore, Md. (1928).

<sup>10</sup> I. M. Kolthoff and J. J. Vleeschhouwer, *Biochem. Z.* 189, 191 (1927).

<sup>11</sup> I. M. Kolthoff and J. J. Vleeschhouwer, *Biochem. Z.* 179, 410-3 (1926).

to constant weight at 160°. Dissolve 5.30 grams in water and dilute to 1 liter.

**0.1 M Citric Acid.** The citric acid should give a clear solution in water, show no test for chloride or sulfate and give practically no ash. To purify, recrystallize the commercial grade 1 or 2 times from water. Dry to constant weight over hydrated sodium bromide,  $\text{NaBr} \cdot 2\text{H}_2\text{O}$ .

To check the amount of water of hydration dry at 20 to 30 mm. pressure at 70°. The acid should remain colorless and lose  $8.58 \pm 0.1$  per cent of moisture.

As buffer dissolve 21.008 grams in water and dilute to 1 liter. As a further check it is advisable to titrate a portion of the solution with 0.2 N barium hydroxide solution to a distinct red with phenolphthalein.

**Buffer for Wide Range**—McIlvaine<sup>12</sup> has employed 0.2 M disodium phosphate and 0.1 M citric acid for producing a series of buffers covering the range of pH 2.2–8.0.

**Manipulation**—It is convenient to prepare 200 cc. of each buffer at a time. Place in 250 cc. bottles. Perforate the stoppers with an opening which will just take a 10 cc. pipet and leave the pipet in the stopper. If the bottles are to stand for any length of time cover the pipets to prevent collection of dust.

**Buffer Mixtures**—By proper admixture of buffer solutions according to Tables 11-18 buffers of any value from pH 1.0–12.0 can be prepared. As these tables overlap, buffers from one set can be compared with another to insure accuracy.

TABLE 11. BUFFER MIXTURES FOR pH 1.0–2.2

pH	cc. of 0.2M Potassium Chloride	cc. of 0.2M Hydrochloric Acid	Dilute to
1.0	0.00	59.50	100 cc.
1.1	2.72	47.28	100 cc.
1.2	12.45	37.55	100 cc.
1.3	20.16	29.84	100 cc.
1.4	26.30	23.70	100 cc.
1.5	31.18	18.82	100 cc.
1.6	35.03	14.95	100 cc.
1.7	38.12	11.88	100 cc.
1.8	40.57	9.43	100 cc.
1.9	42.51	7.49	100 cc.
2.0	44.05	5.95	100 cc.
2.1	45.27	4.73	100 cc.
2.2	46.24	3.76	100 cc.

<sup>12</sup> T. C. McIlvaine, *J. Biol. Chem.* 49, 183-6 (1921).

TABLE 12. BUFFER MIXTURES FOR pH 2.2-3.8

pH	cc. of 0.2M Potassium Acid Phthalate	cc. of 0.2M Hydrochloric Acid	Dilute to
2.2	50	46.60	200 cc
2.4	50	39.60	200 cc
2.6	50	33.00	200 cc
2.8	50	26.50	200 cc
3.0	50	20.40	200 cc
3.2	50	14.80	200 cc
3.4	50	9.95	200 cc
3.6	50	6.00	200 cc
3.8	50	2.65	200 cc.

TABLE 13. BUFFER MIXTURES FOR pH 4.0-6.2

pH	cc. of 0.2M Potassium Acid Phthalate	cc. of 0.2M Sodium Hydroxide	Dilute to
4.0	50	0.40	200 cc.
4.2	50	3.65	200 cc.
4.4	50	7.35	200 cc
4.6	50	12.00	200 cc
4.8	50	17.50	200 cc.
5.0	50	23.65	200 cc
5.2	50	29.75	200 cc.
5.4	50	35.25	200 cc
5.6	50	39.70	200 cc.
5.8	50	43.10	200 cc
6.0	50	45.40	200 cc
6.2	50	47.00	200 cc

TABLE 14. BUFFER MIXTURES FOR pH 5.8-8.0

pH	cc. of 0.2M Monopotassium Phosphate	cc. of 0.2M Sodium Hydroxide	Dilute to
5.8	50	3.66	200 cc
6.0	50	5.64	200 cc
6.2	50	8.55	200 cc
6.4	50	12.60	200 cc
6.6	50	17.74	200 cc
6.8	50	23.60	200 cc
7.0	50	29.54	200 cc
7.2	50	34.90	200 cc
7.4	50	39.34	200 cc
7.6	50	42.74	200 cc
7.8	50	45.17	200 cc
8.0	50	46.85	200 cc

TABLE 15. BUFFER MIXTURES FOR pH 7.8-10.0

pH	cc. of 0.2 <i>M</i> Boric Acid 0.2 <i>M</i> Potassium Chloride	cc. of 0.2 <i>M</i> Sodium Hydroxide	Dilute to
7.8	50	2.65	200 cc.
8.0	50	4.00	200 cc.
8.2	50	5.90	200 cc.
8.4	50	8.55	200 cc.
8.6	50	12.00	200 cc.
8.8	50	16.40	200 cc.
9.0	50	21.40	200 cc.
9.2	50	26.70	200 cc.
9.4	50	32.00	200 cc.
9.6	50	36.85	200 cc.
9.8	50	40.80	200 cc.
10.0	50	43.90	200 cc.

TABLE 16. BUFFER MIXTURES FOR pH 9.2-11.0

pH	cc. of 0.05 <i>M</i> Sodium Carbonate	cc. of 0.05 <i>M</i> Borax
9.2	0.00	100.00
9.4	35.70	64.30
9.6	55.50	44.50
9.8	66.70	33.30
10.0	75.40	24.60
10.2	82.15	17.85
10.4	86.90	13.10
10.6	91.50	8.50
10.8	94.75	5.25
11.0	97.30	2.70

TABLE 17. BUFFER MIXTURES FOR pH 11.0-12.0

pH	cc. of 0.1 <i>M</i> Disodium Phosphate	cc. of 0.1 <i>M</i> Sodium Hydroxide	Dilute to
11.0	25	4.13	50 cc.
11.2	25	6.00	50 cc.
11.4	25	8.67	50 cc.
11.6	25	12.25	50 cc.
11.8	25	16.65	50 cc.
12.0	25	21.60	50 cc.

TABLE 18. BUFFER MIXTURES FOR pH 2.2-8.0

pH	cc. of 0.2 <i>M</i> Disodium Phosphate	cc. of 0.1 <i>M</i> Citric Acid
2.2	0.40	19.60
2.4	1.24	18.76
2.6	2.18	17.82
2.8	3.17	16.83
3.0	4.11	15.89
3.2	4.94	15.06
3.4	5.70	14.30
3.6	6.44	13.56
3.8	7.10	12.90
4.0	7.71	12.29
4.2	8.28	11.72
4.4	8.82	11.18
4.6	9.35	10.65
4.8	9.86	10.14
5.0	10.30	9.70
5.2	10.72	9.28
5.4	11.15	8.85
5.6	11.60	8.40
5.8	12.09	7.91
6.0	12.63	7.37
6.2	13.22	6.78
6.4	13.85	6.15
6.6	14.55	5.45
6.8	15.45	4.55
7.0	16.47	3.53
7.2	17.39	2.61
7.4	18.17	1.83
7.6	18.73	1.27
7.8	19.15	0.85
8.0	19.45	0.55

**Indicator Solutions**—There are four important classes of indicators; phthaleins, sulfophthaleins, nitrophenols and azo compounds.<sup>13</sup> Each class has characteristic extinction curves. Clark<sup>14</sup> lists 185 colorimetric indicators. Many of these are valueless, yet they are a selection of those recorded as used at some time and are only a small part of the number

<sup>13</sup> A. Thiel and R. Diehl, *Sitzb. Ges. Beförd. ges. Naturw. Marburg* 64, 79-109 (1929).

<sup>14</sup> William Mansfield Clark, "Determination of Hydrogen Ions," 3rd ed., pp. 76-90. Williams and Wilkins Co., Baltimore, Md. (1928).

available. As an example, one article <sup>14a</sup> lists 33 derivatives of dinitroanilines usable as indicators.

Three well selected tables of indicators are available and are given here, although only one will be specifically discussed. The first is that of Sorensen given in Table 20. The second is a set of one-color indicators selected by Michaelis <sup>15</sup> and given in Table 19. The range above pH 10.0 has been fairly well covered by a series of indicators developed in the laboratory of an equipment manufacturer.<sup>16</sup> They are included here under their commercial brands because of the availability of data on their use. The third table is, therefore, a selection of indicators of Clark and Lubs,<sup>2</sup> amplified by Cohen and further amplified by these proprietary indicators over a range not otherwise covered.

Comparison of the Clark and Lubs indicators with those of Michaelis has shown differences of 0.1–0.2 pH.<sup>18</sup> The differences are in unbuffered solution and due to the varying alkaline content of the dye or to variations in its constitution.<sup>21</sup> Bromothymol blue seems particularly susceptible to variation. *p*-Nitrophenol is quite suitable for unbuffered solutions.

Numerous indicators have been examined for high pH ranges and found unsatisfactory.<sup>22</sup> The most promising as given in Table 21 are supplemented by Table 22.

TABLE 19. MONOCHROMATIC INDICATORS OF MICHAELIS

Picric acid.....	colorless	0.0– 1.3 yellow
2, 4-dinitrophenol.....	colorless	2.0– 4.7 yellow
$\alpha$ -dinitrophenol		
2, 6-dinitrophenol.....	colorless	1.7– 4.4. yellow
$\beta$ -dinitrophenol		
2, 5-dinitrophenol.....	colorless	4.0– 6.0 yellow
$\gamma$ -dinitrophenol		
<i>m</i> -nitrophenol.....	colorless	6.3– 9.0 yellow
<i>p</i> -nitrophenol.....	colorless	4.7– 7.9 yellow
Phenolphthalein.....	colorless	8.5–10.5 red
Alizarin yellow GG.....	colorless	10.0–12.0 yellow
Salicyl yellow		

<sup>14a</sup> Henry Wenker, *Ind. Eng. Chem., Anal. Ed.* 7, 40-1 (1935).

<sup>15</sup> Cf. J. M. deCastro Marcal, *Rev. brasil med. pharm.* 8, 211-28 (1933).

<sup>16</sup> Private communication from F. R. McCrumb.

<sup>18</sup> E. Ramann and H. Sallinger, *Z. anal. Chem.* 63, 292 (1923).

<sup>21</sup> H. T. Stern, *J. Biol. Chem.* 65, 677-81 (1925).

<sup>22</sup> F. R. McCrumb and W. R. Kenny, *Ind. Eng. Chem., Anal. Ed.* 1, 44-6 (1929).



TABLE 20. INDICATORS OF SORESENSEN

	Composition of Test Solution	Useful Range pH	Sensitivity to Neutral Salts	USEFULNESS IN PRESENCE OF			Stability on Standing
				True proteins	High conc. of products of proteolysis	Chloroform and toluene	
Methyl violet 6B.....	0.01-0.05 per cent aqueous	y 0.1- 3.2 v	high	fair	good	with chloroform not, with toluene useful	acid solutions fade
Mauve.....	0.01-0.05 per cent aqueous	0.1- 2.9	high	fair	good	as above	as above
Benzene-azo-diphenylamine.....	0.01 gram in 1 cc. <i>N</i> HCl + 50 cc. alcohol + 49 cc. water	p 1.2- 2.1 y	low	not	fair	not	moderate
Tropaeolin OO.....	0.01 per cent aqueous	r 1.4- 2.6 y	low	not	fair	good	good
Metanil yellow.....	0.01 per cent aqueous	r 1.2- 2.3 y	low	not	fair	good	good
Benzene-azo-benzylaniline.....	0.02 gram in 1 cc. 0.1 <i>N</i> HCl + 50 cc. alcohol + 49 cc. water	p 2.3- 3.3 y	low	not	good	not	moderate
<i>p</i> -benzenesulfonic acid-azo-benzylaniline.....	0.01 per cent aqueous	r 1.9- 3.3 y	low	not	fair	good	good
<i>p</i> -benzenesulfonic acid-azo- <i>m</i> -chlorodimethylaniline.....	0.01 per cent aqueous	r 2.6- 4.0 y	low	not	fair	good	good
Benzene-azo-dimethylaniline.....	0.01 gram in 0.1 cc. 0.1 <i>N</i> HCl + 80 cc. alcohol + 20 cc. water	r 2.9- 4.0 y	low	not	good	not	moderate
Methyl orange.....	0.01 per cent aqueous	r 3.1- 4.4 y	low	not	fair	good	good
Benzene-azo- $\alpha$ -naphthylamine.....	0.01 gram in 0.4 cc. 0.1 <i>N</i> HCl + 30 cc. alcohol + 70 cc. water	r 3.7- 5.0 y	low	not	good	not	moderate

S.C. = useful in special cases. b = blue; br = brown; c = colorless; o = orange; p = pink; r = red; v = violet; y = yellow.

TABLE 20. INDICATORS OF SORENSEN—Continued

	Composition of Test Solution	Useful Range pH	Sensitivity to Neutral Salts	USEFULNESS IN PRESENCE OF			Stability on Standing
				True proteins	High conc. of products of proteolysis	Chloroform and toluene	
<i>p</i> -benzenesulfonic acid-azo- $\alpha$ -naphthylamine.....	0.01 gram in 60 cc. alcohol + 40 cc. water	r 3.5- 5.7 y	low	not	good	good	good
Methyl red.....	0.02 gram in 60 cc. alcohol + 40 cc. water	r 4.2- 6.3 y	low	S.C.	good	good	moderate
<i>p</i> -nitrophenol.....	0.04 gram in 6 cc. alcohol + 94 cc. water	c 5.0- 7.0 y	moderate	good	good	good	good
Neutral red.....	0.01 gram in 50 cc. alcohol + 50 cc. water	r 6.8- 8.0 y	low	S.C.	good	S.C.	good
Rosolic acid.....	0.04 gram in 40 cc. alcohol + 60 cc. water	br 6.9- 8.0 r	low	fair	good	fair	good
Tropaeolin OOO No. 1.	0.01 per cent aqueous	v 7.6- 8.9 p	low	good	good	good	good
<i>p</i> - $\alpha$ -naphtholphthalein.	0.1 gram in 150 cc. alcohol + 100 cc. water	y 7.3- 8.7 b	moderate	S.C.	good	good	fair
Phenolphthalein.....	0.05 gram in 50 cc. alcohol + 50 cc. water	c 8.3-10.0 r	moderate	S.C.	good	good	good—fades in strong alkali
Thymolphthalein.....	0.04 gram in 50 cc. alcohol + 50 cc. water	c 9.3-10.5 b	moderate	S.C.	good	good	fades in moderate alkali
Alizarin yellow R.....	0.01 per cent aqueous	y 10.4-12.1 y			good		good
Tropaeolin O.....	0.01 per cent aqueous	y 11.1-12.7 o			fair		good

S.C. = useful in special cases. b = blue; br = brown; c = colorless; o = orange; p = pink; r = red; v = violet; y = yellow.

TABLE 21. INDICATORS OF CLARK, AND LUBS,<sup>20</sup> COHEN,<sup>20</sup> AND LA MOTTE  
CHEMICAL PRODUCTS CO.

Common Name	Molecular Weight	cc. 0.01 <i>N</i> NaOH per 0.1 g. Indicator	pK	Range <i>pH</i>	Color Change Acid - Alkaline	<i>pH</i> required for full acid color	<i>pH</i> required for full alkaline color	Absorption Maximum	
								Acid <i>mμ</i>	Alk. <i>mμ</i>
<i>m</i> -Cresol purple.....	382	26.2	1.51	1.2-2.8	red-yellow	conc. HCl	6	533	592
Thymol blue.....	466	21.5	1.5	1.2-2.8	red-yellow	conc. HCl	6	544	617
Bromophenol blue.....	669	14.9	3.98	3.0-4.6	yellow-blue	0	7		573
Bromocresol green.....	698	14.3	4.67	3.8-5.4	yellow-blue	1	8		574
Chlorophenol red.....	423	23.6	5.98	4.8-6.4	yellow-red	2	9		591
Bromophenol red.....	512	19.5	6.16	5.2-6.8	yellow-red	3	10		617
Bromocresol purple.....	540	18.5	6.3	5.2-6.8	yellow-purple	3	10		558
Bromothymol blue.....	624	16.0	7.0	6.0-7.6	yellow-blue	4	11		580
Phenol red.....	354	28.2	7.9	6.8-8.4	yellow-red	5	11		596
Cresol red.....	382	26.2	8.3	7.2-8.8	yellow-red	5	11		
<i>m</i> -Cresol purple.....	382	26.2	8.32	7.4-9.0	yellow-purple	5	11		
Thymol blue.....	466	21.5	8.9	8.0-9.6	yellow-blue	6	12		
Cresolphthalein.....			9.4	8.2-9.8	colorless-red	6	12		
La Motte purple				9.6-11.2	blue-violet				
Nitro yellow				10.0-11.6	colorless-deep yellow				
Sulfo orange				11.0-12.6	pale yellow-deep orange				
La Motte violet				12.0-13.6	red-blue				

20 B. Cohen, *U. S. Public Health Repts.*, 41, 3051 (1927).

In Figure 98 are given the ranges and color changes of indicators available from the Eastman Kodak Co. A useful chart of the range of color change of common indicators has been prepared and is shown in Figure 99.<sup>23</sup>

Tetrabromophenoltetrabromosulfonephthalein<sup>24</sup> has been proposed to replace bromophenol blue, under the name of tetrabromophenol blue. It shows a color change from yellow to blue but is free from dichromatism.

TABLE 22. INDICATORS FOR HIGH pH VALUES

Common Name	Chemical Name	Color Change			
Alizarin Yellow GG	<i>m</i> -Nitrobenzeneazosalicylic acid	Lemon Yellow	10.0	Yellow	12.0
Alizarin Yellow R	<i>p</i> -Nitrobenzeneazosalicylic acid	Yellow	10.0	Orange	12.0
Tropeolin O	<i>p</i> -Sulfobenzeneazoresorcin	Yellow	11.0	Orange	13.0
Azo blue	Ditolyldisazo-bis- $\alpha$ -naphthol-4-sulfonic acid	Violet	10.0	Purple	11.0
	$\alpha$ Naphthol benzein	Yellow	9.0	Green	10.6

Pinachrom is an excellent one color indicator<sup>25</sup> for the range 5.8 to 7.8. It is recommended for tap water and distilled water. At low electrolyte content the salt error is negligible. At high salt concentrations the reaction indicated is too acid. For use by the balancing method the pK value of the indicator must be known. The values are given with the necessary tables for the Clark and Lubs-Cohen series in a later chapter.

**pH of Indicator Solutions**—Unless the sample solution is well buffered, dilution by the indicator solution and reaction of the indicator with hydrogen-ion or hydroxyl-ion may affect the pH.

For slightly buffered solutions therefore the pH of the indicator solution must be adjusted to about the middle of the transition range.<sup>26</sup> In exceedingly dilute solutions or in weakly buffered solutions of high neutral salt content a new definition of colorimetric pH must therefore be written. It is that the pH of the solution is that of the indicator solution which does not change color when added to the unknown.<sup>27</sup> Salt and protein errors must still be given consideration. Because of the extreme care required and the great detail of the procedure, the original reference must be consulted. If the pH of the indicator solution is within 0.5 pH of that being determined, substantial accuracy is obtained.

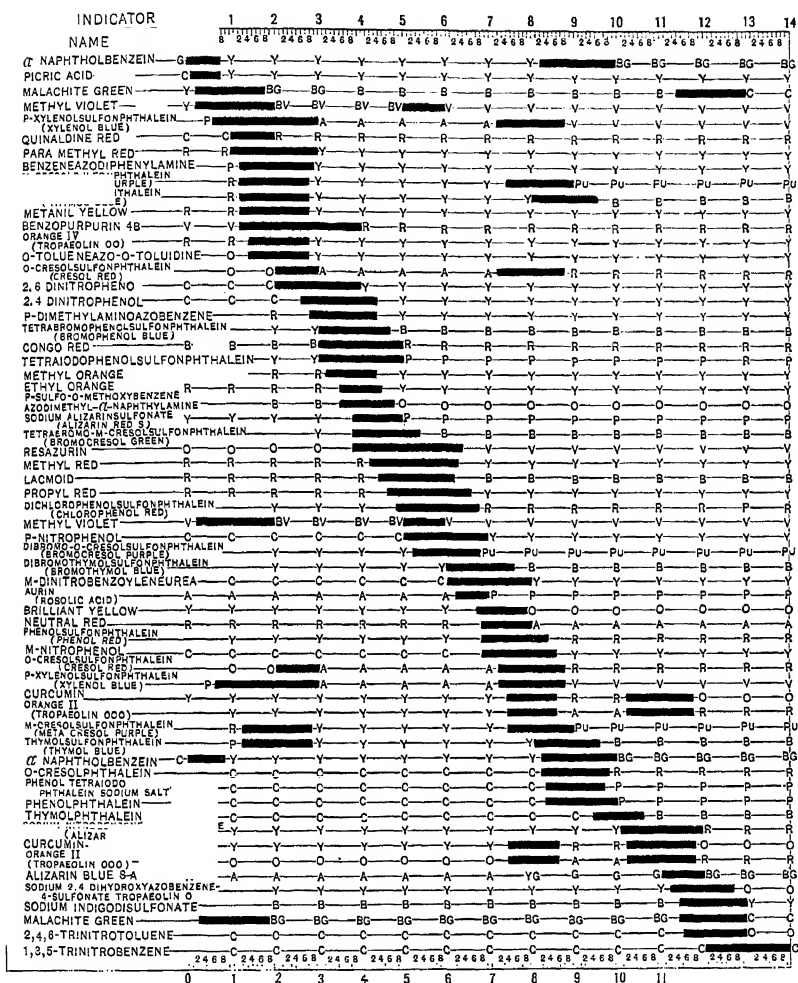
<sup>23</sup> Clarke E. Davis and Henry M. Salisbury, *Ind. Eng. Chem., Anal. Ed.* 1, 92 (1929).

<sup>24</sup> Wilton C. Harden and Nathan L. Drake, *J. Am. Chem. Soc.* 51, 562-6, 2278-9 (1929).

<sup>25</sup> I. M. Kolthoff, *J. Am. Chem. Soc.* 50, 1604-8 (1928).

<sup>26</sup> W. H. Pierre and J. F. Fudge, *J. Am. Chem. Soc.* 50, 1254-62 (1928).

<sup>27</sup> S. F. Acree and Edna H. Fawcett, *J. Bact.* 17, 163-204 (1929); *Ind. Eng. Chem., Anal. Ed.* 2, 78-85 (1930).



A—Amber  
B—Blue  
C—Colorless  
BG—Blue Green

BV—Blue Violet  
G—Green  
O—Orange  
P—Pink

Pu—Purple  
R—Red  
V—Violet  
Y—Yellow

The pH ranges shown are approximations and are intended to aid in selecting the proper indicator.

Fig. 98

Hydrogen-Ion Concentration Ranges (pH) and Color Changes of Indicators.

(Courtesy of Eastman Kodak Co.)

This would not apply to a case like distilled water, but neither would the other details of the usual technique. Fawcett and Acree have not only developed a method of obtaining colorimetric pH values with great accuracy, but have at the same time offered a method for determination of salt or protein error, which will doubtless be the basis of further research.

The isohydric indicator solutions can also be prepared by purification

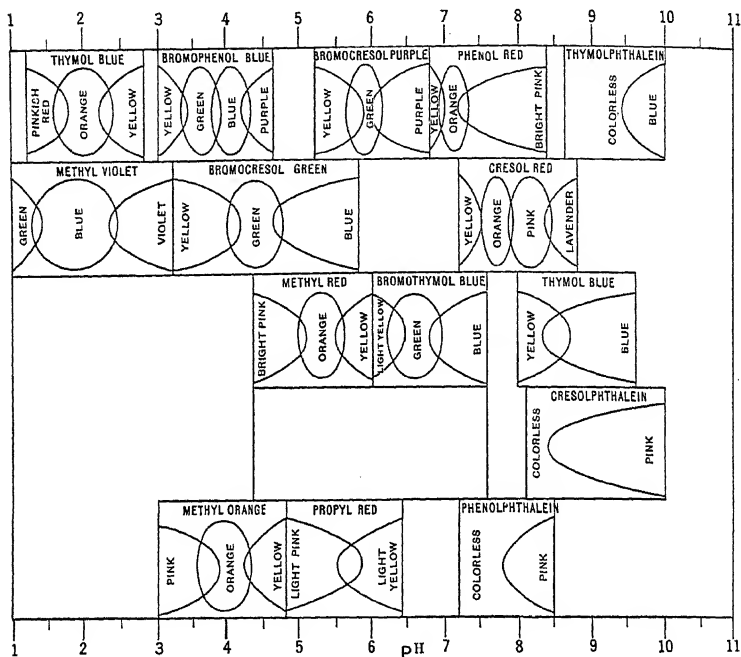


FIG. 99

pH Range of Common Indicators

of the indicator until conductimetric titration gives theoretical results and by calculation from the ionization constant of the indicator to the amount of sodium hydroxide necessary for solutions of definite pH. Estimations with isohydric indicators can also be conveniently carried out by use of a series of indicators having a range of pH values, not necessarily accurately known.<sup>28</sup> If the pH value determined remains constant when an additional amount of indicator is added the indicator is isohydric. This is a simpler technique.

<sup>28</sup> I. M. Kolthoff and Tohru Kameda, *J. Am. Chem. Soc.* 53, 825-32 (1931).

**Universal Indicator**—For rough estimation the so-called Universal Indicator undoubtedly has a use. A proprietary one of British Drug Houses<sup>29</sup> covers the range of 2 to 12. One<sup>30</sup> such indicator has the following composition: phenolphthalein 100 mg., methyl red 200 mg., dimethylaminoazobenzene 300 mg., bromothymol blue 400 mg., thymol blue 500 mg. For preparation dissolve in 500 cc. of absolute alcohol and add 0.1 *N* sodium hydroxide solution until the red disappears and

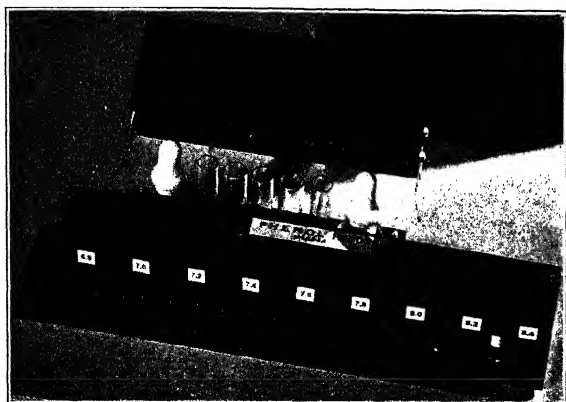


FIG. 100

Slide Comparator for Series of Liquid Standards. (Courtesy of Holmes I. Mettee, Baltimore, Md.)

the solution indicates a pH of 6.0 by a yellow color. The color changes are as follows:

Color	Red	Orange	Yellow	Green	Blue
pH	2.0	4.0	6.0	8.0	10.0

Another<sup>31</sup> contains 0.1 per cent solutions of the following: 15 cc. dimethyl yellow, 5 cc. methyl red, 20 cc. bromothymol blue, 20 cc. phenolphthalein, 20 cc. thymolphthalein. The following colors are obtained.

Color	Rose	Red-Orange	Orange	Yellow-Orange	Lemon-Yellow
pH	1.0	3.0	4.0	5.0	6.0

<sup>29</sup> E. B. R. Prideaux and A. T. Ward, *J. Chem. Soc.* 125, 426-30 (1924).

<sup>30</sup> E. Bogen, *J. Am. Med. Assoc.* 89, 199 (1927).

<sup>31</sup> I. M. Kolthoff, *Pharm. Weekblad* 66, 67-70 (1929).

Color	Yellow Green	Green	Blue- Green	Violet
pH	7.0	8.0	9.0	10.0
	70                      n 00, 100 mg. methyl			

Color	Orange- Red	Red- Orange	Orange	Yellow- Orange	Orange- Yellow	Yellow	Green- Yellow
pH	2.0	3.0	4.0	5.0	6.0	6.5	7.0

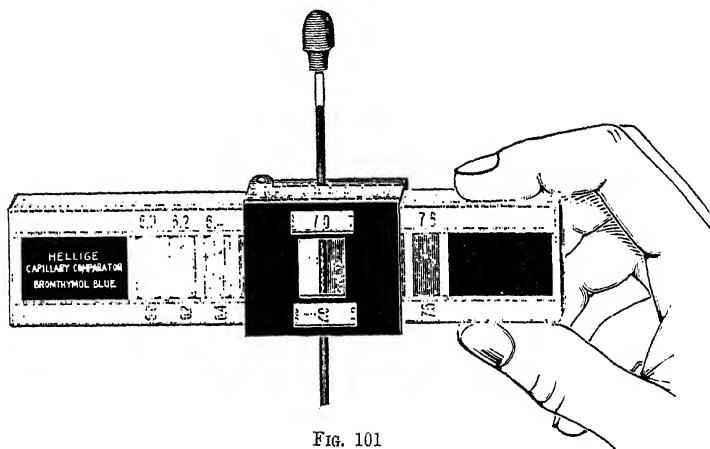


FIG. 101

Hellige Capillary Comparator. (Courtesy of Hellige, Inc.)

Color	Green	Green- Blue	Violet-Blue to Blue-Violet	Violet	Violet to Violet-Red	Violet- Red
pH	8.0	9.0	9.5	10.0	11.0	12.0

mate pH can be obtained to be amplified by over a small range. For many rough, preliminary Universal Indicators is sufficiently accurate.

### Glass Stand

glass in place of available,<sup>33</sup> but are not widely used.



## CHAPTER LXVI

### HYDROGEN-ION—SAMPLES

THIS subject is so broad that of necessity many kinds of samples are not mentioned. Only a few of the special forms can be detailed because of limitations of space. By analogy the methods given can be applied to many other forms of sample, with or without preliminary treatment.

**Water and Clear Liquids**—The water or solution is used without treatment. Monochromatic indicators have been recommended for comparison by balancing.<sup>1</sup> Colorimetric pH is suitable for controlling coagulation in water purification, especially if lime has to be added with the aluminum sulfate.<sup>2</sup>

*Water. Alternative Method.*<sup>3</sup> Approximate values can be obtained by adding 0.5 cc. of 0.5 per cent phenolphthalein solution to 100 cc. of water, without comparison with standards. The following table gives the relative comparisons, and must be questioned as to the absolute accuracy.

Color	Estimated pH Equivalent
Indistinguishable from blank . . . . .	7.8
Indistinguishable without comparison with blank . . . . .	8.0
Faintly pink . . . . .	8.2
Pink . . . . .	8.4
Strongly pink . . . . .	8.6
Very strongly pink . . . . .	8.8
Red . . . . .	9.0

**Sea Water**<sup>4</sup>—To every 100 cc. add 4 drops of 2.5 per cent mercuric chloride solution. Leave a small air space in the bottles. Use cresol red as indicator. The sample will keep for 2 weeks at 12° or 2 days at 33°.

<sup>1</sup> Daphne Goulston, *J. Proc. Roy. Soc. (N. S. Wales)* 65, 37-9 (1931).

<sup>2</sup> Perkins Boynton, *W. Va. Eng. Expt. Sta., Tech. Bull.* 3, 103-6 (1930); *Ibid.* 4, 39-41 (1931).

<sup>3</sup> Abel Wolman and Frank Hannan, *Chem. and Met. Eng.* 25, 503-6 (1921).

<sup>4</sup> O. Gormez Ibanez, *J. Marine Biol. Assoc.* 17, 483-8 (1931).

**Acetone-Water Mixtures**<sup>5</sup>—Indicators give consistent values in such a mixture made acid or alkaline with hydrochloric acid or sodium hydroxide solution, within an accuracy of 0.1 pH when compared with a quinhydrone electrode. The hydrogen electrode becomes poisoned quickly. The indicators designed for use in water are suitable but require a special series of buffers since the color changes of the indicators in acetone-water mixtures are not the same as in water. The original must be consulted for full details.

**Sugar Solutions**<sup>6</sup>—Dilute a solution of sugar to  $22 \pm 0.5^\circ$  Bé. This concentration is a compromise. The pH is less affected by dilution the more concentrated the solution used. The solution must be diluted to such an extent that it can be measured readily and mixed with the indicator. Compare with the methyl red series of indicators after suitable treatment.

**Molasses**<sup>7</sup>—Dilute the sample with 9 volumes of distilled water. The results agree within experimental error with those obtained by electro-metric methods.

**Sulfonated Oil**<sup>8</sup>—Estimation of pH is difficult due to cloudiness when dispersed in water. The value decreases with dilution and increases when mineral oil is added. Values agree with those with the glass and quinhydrone electrodes.

**Soil**<sup>9</sup>—Water used for extraction of soil acidity should be as free from carbon dioxide as possible. Distilled water is best, although high grade ground water may be used. Shake 4 to 5 grams of soil from the roots of living plants into an empty container. Add 15 cc. of water. Shake and let the solid matter settle. Decant or pipet the more or less clear liquid and use as sample.

Another proposal<sup>10</sup> is to shake 6 grams of soil with 12 cc. of *N* potassium chloride solution and 6 drops of a 0.2 per cent solution of bromo-

<sup>5</sup> F. W. Cray and G. M. Westrip, *Trans. Faraday Soc.* 21, 326-7 (1925).

<sup>6</sup> Otto A. Sjostrom, *J. Ind. Eng. Chem.* 14, 941-3 (1922).

<sup>7</sup> P. Dopter, *Bull. soc. chim. biol.* 12, 1031-2 (1930).

<sup>8</sup> D. Burton and G. F. Robertshaw, *J. Intern. Soc. Leather Trades Chem.* 18, 19-22 (1934).

<sup>9</sup> E. T. Wherry, *J. Washington Acad. Sci.* 10, 217-23 (1920).

<sup>10</sup> H. W. Kerr and N. J. King, *Proc. 4th Congr. Intern. Soc. Sugar Cane Tech. Bull.* No. 110 (1932).

cresol green and compare with buffers after 15 minutes. If the solution is too turbid, prepare a larger sample, filter through paper and test successive portions of the filtrate until two give the same pH. Avoid absorption of carbon dioxide as far as possible.

Large deviations occur by the Wulff and the Tödt<sup>11,12</sup> procedures when the calcium carbonate content exceeds 1.5 per cent.<sup>13</sup> Colloidal clay as an ultrafilter is satisfactory.<sup>14</sup> It is preferable to centrifuge and avoid filtration.<sup>15</sup> The solution may be cleared by shaking with barium sulfate prepared for X-ray work.<sup>16,17</sup> Loams give satisfactory results by this method but some clays and volcanic soils require 30 to 40 per cent of the weight of the soil in barium sulfate<sup>18</sup> to clarify them.<sup>19</sup> In the latter cases, agreement with the quinhydrone method is poor. Dialysis of 1 part of soil in 2 parts of water through parchment for 24 hours has been found satisfactory in many cases.<sup>20</sup> Some large deviations occurred.

**Milk—General.** Determination of the pH is difficult due to turbidity of the sample. Addition of bromocresol purple directly to the milk has been proposed.<sup>21,22</sup> Comparison was with normal milk, and with normal milk to which known amounts of acid or alkali had been added. The value of the method is hampered by lack of definite color standards and by the opacity of the milk. The pH obtained by 7.5 minutes' dialysis of 1 cc. of milk against 2 cc. of neutral distilled water has been proposed.<sup>23</sup> Dialysis of 1 cc. of milk against 2 cc. of 0.8 per cent neutral salt solution for 5 minutes is another proposed procedure.<sup>24</sup> A 5 minute dialysis of 5 cc. of milk against 5 cc. of 0.9 per cent salt solution is suggested.<sup>25</sup> This

<sup>11</sup> G. Gollnow, *Chem.-Ztg.* 57, 374-5 (1933).

<sup>12</sup> G. Gollnow, *Rieten Biet.* 6, 159-61 (1931).

<sup>13</sup> Kurt Utescher, *Z. Pflanzenernähr., Düngung Bodenk.* 23A, 381-92 (1932).

<sup>14</sup> W. H. Pierre, *Soil Science* 20, 285-305 (1925).

<sup>15</sup> Felix G. Gustafson, *Ecology* 9, 360-3 (1928).

<sup>16</sup> Istvan Kühn, *Z. Pflanzenernähr., Düngung Bodenk.* 18A, 309-14 (1930).

<sup>17</sup> Cf. S. Goy and O. Roos, *Z. Pflanzenernähr., Düngung Bodenk.* 23A, 63-8 (1930).

<sup>18</sup> L. D. Baver and C. J. Rehling, *Ind. Eng. Chem., Anal. Ed.* 2, 338 (1930).

<sup>19</sup> Alfred J. Parker and L. S. Spackman, *New Zealand J. Sci. Tech.* 13, 48-9 (1931).

<sup>20</sup> W. A. J. Oosting, *Chem. Weekblad* 26, 618-9 (1929).

<sup>21</sup> J. C. Baker and L. L. Vanslyke, *J. Biol. Chem.* 40, 357-373 (1919).

<sup>22</sup> J. C. Baker and R. S. Breed, *J. Biol. Chem.* 43, 221 (1920).

<sup>23</sup> E. W. Schultz and L. R. Chandler, *J. Biol. Chem.* 46, 129 (1921).

<sup>24</sup> E. W. Schultz, A. Marx and H. J. Beaver, *J. Dairy Sci.* 4, 1 (1921).

<sup>25</sup> B. Kramer and C. H. Greene, *J. Biol. Chem.* 46, 42 (1921).

parallels a method for blood and is believed to be reliable. Yet another proposal is to dialyse until equilibrium is reached.<sup>26</sup>

Dilutions of 1:15 or 1:20 for milk<sup>27</sup> and 1:5 for cream<sup>28</sup> have been used for comparative results. Casein tends to adsorb the color<sup>29</sup> and precipitation by rennet has value for comparative purposes. Assuming that milk and buffers change to the same extent on dilution, 2 drops of milk have been added to 10 cc. of water and compared with the same number of drops of buffer added to other test tubes.<sup>30</sup> This assumption is incorrect. Comparison of 10-drop samples, 3 mm. deep on opal glass plates has been used to minimize the effect of turbidity.<sup>31</sup>

The methods using dialysis eliminate turbidity and protein error but introduce a Donnan equilibrium, a salt error and a dilution effect. The dilution methods decrease turbidity and are suitable provided the corrections are known. In 1:20 dilution extreme accuracy is not possible but accuracy to 0.1 pII can be attained, the average error being 0.06 pH. Colorimetric results on milk are usually 0.15–1.07 pII unit lower than electrometric.<sup>32</sup> The difference is usually less in fresh milk than in sour milk.

*Whole and Skim Milk.*<sup>34</sup> If casein has started to separate break up the curd thoroughly and mix with the whey before sampling. Pipet 5 cc. into a 100 cc. graduated cylinder and dilute to volume. Pipet 10 cc. of sample into 1 tube and add indicator. Let casein separate if necessary. Compare, using a tube of diluted milk in back of the standard to correct for turbidity. The general procedure is therefore that for colored samples. Apply correction from Table 23.

*Heated Milk.* When heated to boiling without loss of water treat the same as whole milk.

*Whey.* Proceed in the same way as for whole milk. If only part of the casein has separated the correction factors in Table 23 do not apply and the degree of separation of the casein must be estimated.

*Cream.* Obtain skim milk from the cream by centrifuging and determine as above. Assume that the value for the cream is the same as that

i Galletti, *Arch. farmacol. sper.* 54, 229-36 (1932).

own, *J. Lab. Clin. Med.* 9, 239 (1924).

y and V. Weston, *J. Proc. Roy. Soc. N. S. Wal* 57,  
(1924).

obtained for the skim milk. If the cream has soured so that part of the casein is thrown down in centrifuging the correction factors do not apply.

*Powdered Milk.* Add distilled water so that the average total solids, not fat, corresponds to whole milk. Proceed as for milk.

*Evaporated Milk.* Dilute 5 cc. with distilled water to 200 cc. and proceed as for milk. The pH of the evaporated milk before reconstituting can be obtained by subtracting 0.13 pH from the corrected value obtained for the reconstituted milk.

*Sweetened Condensed Milk.* Dilute with water so that the average solids, not fat, corresponds to whole milk. Proceed as for whole milk except that correction factors in Table 23 do not apply. Instead subtract 0.85 pH from the value obtained to get the pH of the reconstituted product or 1.15 pH to get the value for the original product.

**Brewery Products**<sup>35</sup>—Variations in pH in the brewing industry are small and the importance of small changes is great. Some authors state that accuracy to 0.02–0.03 pH is necessary and cannot be attained colorimetrically. Another<sup>36</sup> finds from the well buffered effect in such products that wort may be diluted 5 to 10 times and stout as much as 40 times without serious error. Another<sup>37</sup> finds that thymol blue, bromophenol blue, methyl red and bromothymol blue may be used.

It has been proposed to compare a mixture of buffer, indicator and water with the sample, indicator and water.<sup>38</sup> Various errors include adsorption by colloids and reduction or oxidation of the indicator. The proposed technique may minimize them.

**Bread**<sup>38a</sup>—Swirl 10 grams of finely divided sample with 100 cc. of distilled water at 25°. When the particles are evenly suspended and free from lumps place in a thermostat at 25° for 30 minutes with intermittent swirling to keep them in suspension. Let stand and decant the clear upper layer as sample. Use at once. As indicators use *p*-nitrophenol for pH 5.4–7.0 and 2,5-dinitrophenol for 4.0–5.6. Trouble is encountered with bromocresol purple.

<sup>35</sup> P. Petit, *Brasserie et malterie* 18, 82-6 (1928).

<sup>36</sup> H. L. Hind, *J. Inst. Brewing* 30, 57-60 (1924).

<sup>37</sup> P. Hampshire, *Bull. Bur. Bio-Technology* 3, 55-65 (1921).

<sup>38</sup> P. Kolbach, *Wochschr. Brau.* 49, 81-5 (1932).

<sup>38a</sup> R. J. Clark, *J. Assoc. Official Agr. Chem.* 17, 392-3 (1934).

TABLE 23. CORRECTION FACTORS FOR COLORIMETRIC DETERMINATION OF  
HYDROGEN-ION CONCENTRATION OF MILK AND WHEY  
BY DILUTION METHOD.<sup>34</sup>

Colorimetric reading.	Whole milk and skim milk.		Whey.		Indicator.
	Correction factor.	Original undiluted.	Correction factor.	Original undiluted.	
<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>	
7.4	0.54	6.86			Phenol red.
7.3	0.54	6.76			
7.2	0.54	6.66	0.26	6.94	
7.1	0.54	6.56	0.26	6.84	
7.0	0.54	6.46	0.26	6.74	
6.9	0.54	6.36	0.26	6.64	
6.8	0.54	6.26	0.26	6.54	
6.7	0.54	6.16	0.26	6.44	
6.6	0.54	6.06	0.26	6.34	Bromocresol purple.
6.5	0.54	5.96	0.26	6.24	
6.4	0.54	5.86	0.25	6.15	
6.3	0.54	5.76	0.25	6.05	
6.2	0.54	5.66	0.24	5.96	Chlorophenol red.
6.1	0.53	5.57	0.23	5.87	
6.0	0.52	5.48	0.23	5.77	
5.9	0.50	5.40	0.22	5.68	
5.8	0.48	5.32	0.22	5.58	
5.7	0.46	5.24	0.22	5.48	
5.6	0.43	5.17	0.22	5.38	
5.5	0.39	5.11	0.22	5.28	
5.4	0.35	5.05	0.22	5.18	
5.3	0.31	4.99	0.22	5.08	
5.2	0.28	4.92	0.22	4.98	
5.1	0.24	4.86	0.22	4.88	
5.0	0.22	4.78	0.22	4.78	
4.9	0.19	4.71	0.22	4.68	Bromocresol green.
4.8	0.18	4.62	0.22	4.58	
4.7	0.17	4.53	0.22	4.48	
4.6	0.16	4.44	0.22	4.38	
4.5	0.16	4.34	0.22	4.28	
4.4	0.15	4.25	0.22	4.18	Bromophenol blue.
4.3	0.15	4.15	0.22	4.08	
4.2(?)	0.15	4.05	0.22	3.98	
4.1(?)	0.16	3.94	0.22	3.88	
4.0(?)	0.16	3.84	0.22	3.78	

The product is diluted 1 to 19 with distilled water and the colorimetric determination made in the conventional way with turbidity blank. The colorimetric pH reading is located in the first column, then the pH of the undiluted milk or whey is found in the corresponding row.

**Meat Extract** <sup>38b</sup>—Extract 10 grams of fat-free finely ground meat for 15 minutes with 100 cc. of boiled distilled water. Filter and use the filtrate as sample. The pH is 6.2 for meat of good quality except for pork which gives 6.4.

**Crackers**—Grind in a mortar to a fine meal. Digest 10 grams <sup>39</sup> or 15 grams <sup>40</sup> with 100 cc. of distilled water at room temperature for 5 to 10 minutes. Filter on cheesecloth and use the filtrate as the sample. For the range above phenol red, cresol red is preferable to thymol blue.

**Gelatine Solution** <sup>41</sup>—The colorimetric method does not agree with other methods of determination but is the only reliable method.

**Glue** <sup>42</sup>—The natural color of even a 1 per cent solution of glue requires that the Walpole technique <sup>43</sup> be used. Compare a 6 cc. sample to which 1 cc. of indicator has been added. Compensate by viewing the standard through 6 cc. of sample to which no indicator has been added and view the sample through a tube of water. The Wulff colorimeter with prepared strips of indicator paper may also be used.

**Soap Solutions**—Experience indicates the lack of reliability of simple estimation of pH in soap solutions. Some indicators are reliable and some are not. If two indicators check as to the pH it is generally reliable. The error is believed to be due to adsorption of indicator by micelles of soap not having the pH of the main solution. Indicators not so adsorbed would give correct readings. The errors are in all cases on the acid side.

**Sulfated Fatty Alcohols** <sup>43a</sup>—Estimation of the pH of solutions of the sulfated fatty alcohols, derivatives of lauryl, oleyl and similar alcohols, may be in error as much as one unit. The error is normally on the acid side and is not due to the pH of sulfated fatty acid.

<sup>38b</sup> C. F. van Oyen, *Z. Fleisch-Milchhyg.* 43, 429-31, 449-53 (1933).

<sup>39</sup> R. T. Bohn and R. J. Martz, *Cereal Chemistry* 3, 183 (1926).

<sup>40</sup> A. H. Johnson and C. H. Bailey, *Cereal Chemistry* 1, 327-410 (1924).

<sup>41</sup> R. J. Hartman and I. F. Fleischer, *J. Phys. Chem.* 36, 1136-42 (1932).

<sup>42</sup> Ernst Goebel, *Kunststoffe u. Leim* 29, 202-8 (1932).

<sup>43</sup> See pp. 11-2.

<sup>43a</sup> J. Edward Jones and Harold L. Jones, *J. Phys. Chem.* 38, 243-4 (1934).

**Textile Assistants**<sup>43b</sup>—To obtain correct results immerse a water-filled cell having a transparent cellophane diaphragm in the solution. Allow to stand until dialysis has adjusted the pH between the inside and outside and use the solution in the cell as sample.

**Unbuffered Solutions**<sup>44</sup>—For pH in unbuffered solutions the liquid must be protected from atmospheric carbon dioxide. The solution of the indicator used must have been adjusted to the pH of the solution to be examined.<sup>45</sup> Such indicators are called adjusted or isohydric. This can be done conveniently by using the indicator solution at different pH values, not necessarily accurately known. If the pH after adding different amounts of indicator is the same the indicator was isohydric.

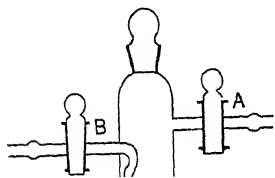


FIG. 102  
Apparatus for Estimation of  
pH in Unbuffered  
Solutions

Introduce a sample of pure water into the Pyrex cell, Figure 102. Pass air through soda-lime, sulfuric acid and several wash bottles of pure water, then through B into the Water in the cell. The water is carbon dioxide-free when successive determinations of pH do not show any change. Remove the stopper of the cell and quickly add the indicator with air flowing through the cell. Then after the color is homogeneous close B and A and compare with the same amount of indicator in a similar volume of buffer solution in another cell. After the reading pass more air through and add more indicator. Repeat with a third addition of indicator. The correct indicator mixture will show the same reading with the addition of different volumes of indicator.

For salt solutions or sodium hydroxide solution add the weighed amount of solute to the carbon dioxide-free water and dissolve it in the cell.

**Nutrient Agar**—Nutrient agar is of itself a buffer so that it will stand seven-fold dilution, at which concentration it is liquid, without change of pH.<sup>46</sup> It may then be used as sample by the usual techniques.

<sup>43b</sup> Harold L. Jones and Edward Smith, *Am. Dyestuff Repts.* 23, 423-7 (1934).

<sup>44</sup> I. M. Kolthoff and Tohru Kameda, *J. Am. Chem. Soc.* 53, 825-32 (1931).

<sup>45</sup> E. H. Fawcett and S. F. Acree, *J. Bact.* 17, 163 (1929); *Ind. Eng. Chem., Anal. Ed.* 2, 78 (1930).

<sup>46</sup> Martin W. Lisse, Otto G. Jensen and Ralph P. Tittsler, *J. Bact.* 21, 383-94 (1931).



**Biological Samples** <sup>47</sup>—Errors are introduced by dilution, temperature correction, salt and protein. The irregularity of the correction factors limits the application of colorimetric methods to those samples of urine, tissue fluids and similar secretions, for which great accuracy is not essential. This criticism should not apply with special techniques such as that of Hastings and Sendroy. <sup>47a</sup>

**Urine** <sup>48</sup>—The method <sup>49</sup> for colored solutions is used. With the indicators phenol red, bromocresol purple and bromocresol green with urine at 1:5 dilution, a correction of  $-0.2$  pH is necessary. About half is for temperature and half for salt and buffer. Comparison with results on undiluted samples at  $38^{\circ}$  showed that no single correction constant would hold and that for accurate results the urine must not be diluted and work must be done at  $38^{\circ}$ . In contrast with this, dilution with a saline diluent of definite pH has been carried out satisfactorily by the balancing method, the determinations being carried out under oil at  $25^{\circ}$ . Correction factors for conversion to  $38^{\circ}$  were phenol red  $0.24$  pH, bromothymol blue  $0.20$  pH, bromocresol purple  $0.20$  pH, bromocresol green  $0.22$  pH. With salt concentrations above  $0.066 M$  the colorimetric value becomes greater than the electrometric. Electrometric values are considered the most accurate <sup>50</sup> in normal urine. Other work <sup>51</sup> indicates that in protein-free urine below pH  $6.5$  all methods are satisfactory, within pH  $6.5$ – $7.5$  the quinhydrone method is best, above pH  $7.5$  only the colorimetric method can be used. Strongly colored urines render the colorimetric method difficult, if not impossible. Decolorizing with animal charcoal is not permissible.<sup>52</sup>

**Gastric Contents** <sup>53</sup>—The treatment varies with the degree of acidity. Below pH  $2.1$  use the clear filtrate directly. Above pH  $2.1$  and below  $3.0$  dialyze the sample against normal salt solution and use the dialysate.

The variations between electrometric and colorimetric methods are rather large. Below pH  $3.0$  the differences are never more than  $0.5$  and

<sup>47</sup> Johs. Baumann, *Z. ges. expatl. Med.* 73, 237-50 (1930).

<sup>47a</sup> See pp. 711-13.

<sup>48</sup> W. Biehler, *Z. physiol. Chem.* 110, 298-306 (1920).

<sup>49</sup> See pp. 715-16.

<sup>50</sup> L. F. Pierce and Clara S. Rice, *J. Lab. Clin. Med.* 17, 1133-45 (1932).

<sup>51</sup> Fritz Mainzer and Werner Eden, *Biochem. Z.* 249, 296-307 (1932); *Klin. Wochschr.* 11, 951-2 (1932).

<sup>52</sup> H. Bohn, *Arch. expatl. Path. Pharmacol.* 140, 118-28 (1929).

<sup>53</sup> George Kahn and Joseph Stokes, Jr., *J. Biol. Chem.* 69, 75-84 (1926).

usually less. Above pH 3.0 differences of 1.0 pH are not uncommon, therefore the electrometric method is preferable.

**Cerebrospinal Fluid**<sup>54</sup>—The sample must never come into contact with the air or it will lose carbon dioxide and give too high a value. A special apparatus is required. To one end of a tube of the same size as is used for the standards is fused a 3-way stopcock. The other end carries a capillary tube. The tube is suitably calibrated, and in the case of the apparatus of McQuarrie and Shohl, is of a special type as shown in Figure 103.

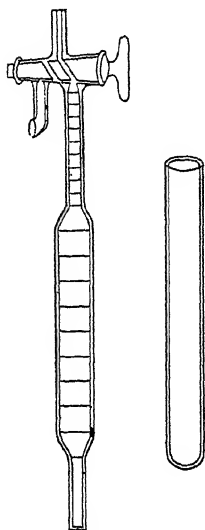


FIG. 103

Apparatus for  
Handling Spinal Fluid  
in pH Determination

Place the indicator in the sampling pipet before taking the sample. Fill the sampling pipet with mercury. Connect the 3-way stopcock to the lumbar puncture needle by a suitably sterilized glass adapter and rubber tubing. Let the fluid escape under its own pressure until the air has been removed from the capillary tube. Then turn the stopcock so that the fluid enters the apparatus. In this operation immerse the sampling bulb in mercury so that the mercury level inside the bulb is at or very slightly above that outside. This is to avoid drawing too great a suction and possible leakage. When sufficient sample has been drawn, turn the stopcock and close the lower capillary with a pinchcock. Immerse the sample with indicator and standards in a water bath at 38° for 5 minutes before comparison. McQuarrie and Shohl using phenol red as indicator found the pH to be 7.35 to  $7.40 \pm 0.02$ .

**Blood, Serum or Plasma**—A special procedure is required, working at 38° with a saline indicator solution.<sup>55</sup>

**Cells and Tissues**<sup>56</sup>—Fill a micro pipet with a warm solution of the indicator containing a suitable concentration of gelatine so that it will set readily on cooling. Puncture the cell or tissue and thus permit the cell contents to diffuse into the micro pipet and the contents of the micro pipet into the cell. The gelatine prevents other mixing. Compare with standards as similar as can be prepared.

<sup>54</sup> I. McQuarrie and A. T. Shohl, *J. Biol. Chem.* **66**, 367-74 (1925).

<sup>55</sup> See pp. 711-13.

<sup>56</sup> T. Peterfi, *Z. wiss. Mikroskop.* **45**, 56-9 (1928).

## CHAPTER LXVII

### HYDROGEN-ION—METHODS OF DETERMINATION

COLORIMETRIC pH is usually accurate to 0.1 unit of pH. That degree of accuracy is readily attainable with some small amount of experience. Procedures have been developed for higher accuracy.

**Standard Tubes**—Colorimetric comparisons of standards for pH are usually made in uniform tubes, for which test tubes without lips are most suitable. A convenient size is 1.5 cm. in diameter and 15 cm. long. For making a selection add 10 cc. of water to a series of tubes which appear to be uniform. A variation of five per cent in height is permissible. Those varying more than that amount from the general average should be discarded.

#### COMPARISON WITH STANDARD BUFFERS

For estimation by this method a series of standard buffers is prepared. The same amount of the same indicator is added to each and to the standard in the same volume. The pH is read by direct comparison, provided the sample is not colored.

**Procedure**—In a series of uniform tubes, usually test tubes, place 10 cc. portions of the various buffer solutions to cover the desired range. To each add 1.0 cc. of standard indicator solution and mix well. By sealing the buffer standards they will keep for some days, the time depending on the particular buffer. Some buffers can be kept for months after the color is developed.

To 10 cc. of the sample add 1 cc. of the appropriate indicator, mix well and compare with the series of standard buffers.

#### ESTIMATION WITHOUT BUFFERS

For a few estimations of pH, preparation of buffers takes a needless amount of time. Gillespie<sup>1</sup> has devised a method which has been modified

---

<sup>1</sup> Louis J. Gillespie, *J. Am. Chem. Soc.* **42**, 742-8 (1920); *Soil Science* **9**, 115-35 (1920).

by Medalie,<sup>2</sup> described in many places<sup>3,4</sup> and further improved by Hatfield.<sup>5</sup> Accurate buffers are not required. Even in the original form the method could be applied by the average water works operator.<sup>6</sup>

This method depends on the fact that the sum of the acid and basic color of an indicator is a linear function over the range of the indicator. Instead of using a buffer, a predetermined amount of indicator and acid may be placed in one tube to give the acid color and a predetermined amount of indicator and base in another tube to give the basic color. The sum of the colors of the two tubes is the same as that which would be given by the indicator with a buffer solution at a calculable pH.

Gillespie used 1 drop of 0.2 per cent sodium hydroxide solution in 5 cc. of water for the alkaline colors and 1 cc. of 0.05 *N* hydrochloric acid diluted to 5 cc for the acid standards. The approximately 0.05 *N* hydrochloric acid was prepared by dilution of 1 cc. of concentrated hydrochloric acid to 240 cc. Similarly approximately 0.2 per cent sodium hydroxide solution was prepared from stick sodium hydroxide. Gillespie's standards lasted only a few hours.

Hatfield uses rough buffers which are not readily affected by slight exposure to air, nor by glass. His colors, with the exception of methyl red, are stable for 4 to 8 weeks if protected from light when not in use.

**Indicators**—The indicators used are the neutralized sulfonphthalein series. The compositions to be used are given in Table 24.

**Buffer Solutions**—Four buffers are required which can be prepared from reagent-grade chemicals without further purification.

TABLE 24. INDICATORS FOR USE WITH GILLESPIE-HATFIELD METHOD

Indicator	pH Range	Weight in Gram	$\mu$	
Bromophenol Blue . . . . .	3.10-5.00	0.02	3.28	250 cc.
Methyl Red . . . . .	4.05-5.95	0.02	...	250 cc.
Bromocresol Purple . . .	5.30-7.20	0.03	8.34	250 cc.
Bromothymol Blue . . . .	6.15-8.05	0.02	3.54	250 cc.
Phenol Red . . . . .	6.75-8.65	0.01	3.10	250 cc.
Cresol Red . . . . .	7.15-9.05	0.02	5.76	250 cc.
Thymol Blue . . . . .	7.85-9.75	0.02	4.76	250 cc.

<sup>2</sup> N. K. Smith, *Bull. Bur. Bio-Technology* 4, 105-7 (1921).

<sup>3</sup> F. J. Watson, *Chem. Eng. Mining Rev.* 19, 381-3 (1927).

<sup>4</sup> H. L. Hind, *J. Inst. Brewing* 30, 57-60 (1924).

<sup>5</sup> William D. Hatfield, *J. Am. Chem. Soc.* 45, 940-3 (1923).

<sup>6</sup> William D. Hatfield, *J. Ind. Eng. Chem.* 14, 1038-40 (1922).

*Acetic Acid.* Dilute 57.7 cc. of glacial acetic acid to 1 liter.

*Monopotassium Phosphate.* Dissolve 7.0 grams of monopotassium phosphate in water and dilute to 1 liter.

*Disodium Phosphate.* Dissolve 18.0 grams of disodium phosphate in water and dilute to 1 liter.

*Sodium Carbonate.* Dissolve 1.0 gram of anhydrous sodium carbonate in water and dilute to 1 liter.

**Standard Color Tubes**—The materials for use in the standard color tubes are outlined in Tables 25–31. Uniform test tubes are required.

To prepare, place in a test tube the desired fraction of a cc. of the indicator solution. To this add the desired buffer to make a total of 1 cc. Add 10 cc. of buffer to each tube. Add 2 to 3 drops of toluene to act as preservative and stopper with paraffined corks.

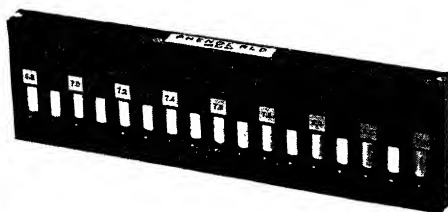


FIG. 104

Series of Liquid Color Standards. (Courtesy of Holmes I. Mettee, Baltimore, Md.)

Toluene will extract the acid color of methyl red and thus shift the apparent color. To avoid this allow the acid color tubes of methyl red to stand loosely stoppered for a few days before paraffining the stoppers. In that time the excess toluene will evaporate.

TABLE 25. BROMOPHENOL BLUE STANDARDS.

pH	Acid Tube		Basic Tube	
	cc. of Indicator	cc. of Acetic Acid	cc. of Indicator	cc. of Disodium Phosphate
3.1	0.10	10.90	0.90	10.10
3.3	0.15	10.85	0.85	10.15
3.5	0.20	10.80	0.80	10.20
3.7	0.30	10.70	0.70	10.30
3.9	0.40	10.60	0.60	10.40
4.1	0.50	10.50	0.50	10.50
4.3	0.60	10.40	0.40	10.60
4.5	0.70	10.30	0.30	10.70
4.7	0.80	10.20	0.20	10.80
4.8	0.85	10.15	0.15	10.85
5.0	0.90	10.10	0.10	10.90

TABLE 26. METHYL RED STANDARDS.

pH	Acid Tube		Basic Tube	
	cc. of Indicator	cc. of Acetic Acid	cc. of Indicator	cc. of Disodium Phosphate
4.05	0.10	10.90	0.90	10.10
4.25	0.15	10.85	0.85	10.15
4.4	0.20	10.80	0.80	10.20
4.6	0.30	10.70	0.70	10.30
4.8	0.40	10.60	0.60	10.40
5.0	0.50	10.50	0.50	10.50
5.2	0.60	10.40	0.40	10.60
5.4	0.70	10.30	0.30	10.70
5.6	0.80	10.20	0.20	10.80
5.75	0.85	10.15	0.15	10.85
5.95	0.90	10.10	0.10	10.90

TABLE 27. BROMOCRESOL PURPLE STANDARDS.

pH	Acid Tube		Basic Tube	
	cc. of Indicator	cc. of Mono-potassium Phosphate	cc. of Indicator	cc. of Sodium Carbonate
5.3	0.10	10.90	0.90	10.10
5.5	0.15	10.85	0.85	10.15
5.7	0.20	10.80	0.80	10.20
5.9	0.30	10.70	0.70	10.30
6.1	0.40	10.60	0.60	10.40
6.3	0.50	10.50	0.50	10.50
6.5	0.60	10.40	0.40	10.60
6.7	0.70	10.30	0.30	10.70
6.9	0.80	10.20	0.20	10.80
7.0	0.85	10.15	0.15	10.85
7.2	0.90	10.10	0.10	10.90

TABLE 28. BROMOTHYMOL BLUE STANDARDS.

pH	Acid Tube		Basic Tube	
	cc. of Indicator	cc. of Mono-potassium Phosphate	cc. of Indicator	cc. of Sodium Carbonate
6.15	0.10	10.90	0.90	10.10
6.35	0.15	10.85	0.85	10.15
6.5	0.20	10.80	0.80	10.20
6.7	0.30	10.70	0.70	10.30
6.9	0.40	10.60	0.60	10.40
7.1	0.50	10.50	0.50	10.50
7.3	0.60	10.40	0.40	10.60
7.5	0.70	10.30	0.30	10.70
7.7	0.80	10.20	0.20	10.80
7.85	0.85	10.15	0.15	10.85
8.05	0.90	10.10	0.10	10.90

TABLE 29. PHENOL RED STANDARDS.

pH	Acid Tube		Basic Tube	
	cc. of Indicator	cc. of Mono-potassium Phosphate	cc. of Indicator	cc. of Sodium Carbonate
6.75	0.10	10.90	0.90	10.10
6.95	0.15	10.85	0.85	10.15
7.1	0.20	10.80	0.80	10.20
7.3	0.30	10.70	0.70	10.30
7.5	0.40	10.60	0.60	10.40
7.7	0.50	10.50	0.50	10.50
7.9	0.60	10.40	0.40	10.60
8.1	0.70	10.30	0.30	10.70
8.3	0.80	10.20	0.20	10.80
8.45	0.85	10.15	0.15	10.85
8.65	0.90	10.10	0.00	10.90

TABLE 30. CRESOL RED STANDARDS.

pH	Acid Tube		Basic Tube	
	cc. of Indicator	cc. of Mono-potassium Phosphate	cc. of Indicator	cc. of Sodium Carbonate
7.15	0.10	10.90	0.90	10.10
7.35	0.15	10.85	0.85	10.15
7.5	0.20	10.80	0.80	10.20
7.7	0.30	10.70	0.70	10.30
7.9	0.40	10.60	0.60	10.40
8.1	0.50	10.50	0.50	10.50
8.3	0.60	10.40	0.40	10.60
8.5	0.70	10.30	0.30	10.70
8.7	0.80	10.20	0.20	10.80
8.85	0.85	10.15	0.15	10.85
9.05	0.90	10.10	0.10	10.90

TABLE 31. THYMOL BLUE STANDARDS.

pH	Acid Tube		Basic Tube	
	cc. of Indicator	cc. of Mono-potassium Phosphate	cc. of Indicator	cc. of Sodium Carbonate
7.85	0.10	10.90	0.90	10.10
8.05	0.15	10.85	0.85	10.15
8.2	0.20	10.80	0.80	10.20
8.4	0.30	10.70	0.70	10.30
8.6	0.40	10.60	0.60	10.40
8.8	0.50	10.50	0.50	10.50
9.0	0.60	10.40	0.40	10.60
9.2	0.70	10.30	0.30	10.70
9.4	0.80	10.20	0.20	10.80
9.55	0.85	10.15	0.15	10.85
9.75	0.90	10.10	0.10	10.90

**Procedure**—To a similar test tube add 10 cc. of sample and 1 cc. of indicator solution. Mix and compare it and a similar tube containing distilled water with the two tubes representing the color. Gillespie com-

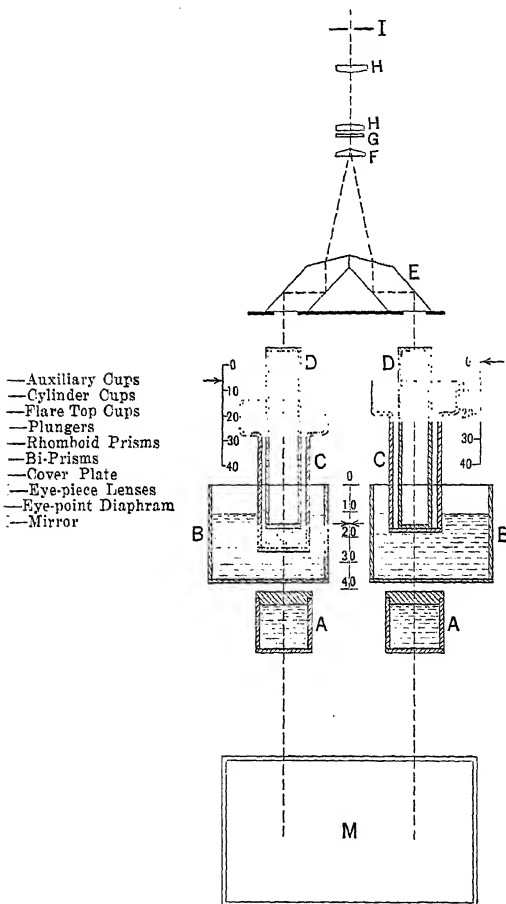


FIG. 105

Modified Duboseq Colorimeter for pH Determination

pared the sample with the standard in a comparator such as is shown in Figure 2.<sup>7</sup> Hatfield constructed one which would have 23 sets of cells so that so much handling of the standards is not necessary.



Instead of using the measurements by cc. as given, the assumption may be made that 1 drop is 0.05 cc. Fractions of cc. can then be taken by drops with a fair degree of accuracy, and if desired, the volumes of sample and standards cut to half those specified.

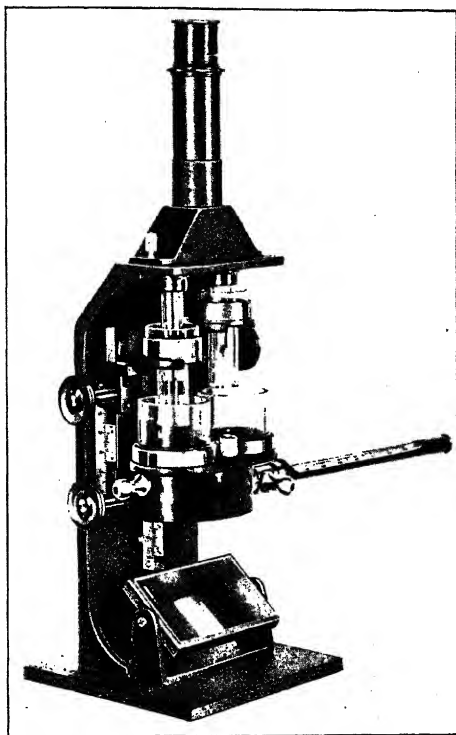


Fig. 106

Duboseq Type of Hydrogen-Ion Colorimeter. (Courtesy of Bausch & Lomb Optical Co.)

#### BALANCING METHOD

For this method a special type of colorimeter is required, in which the total depth of the two standards is maintained at uniformly that of the sample. The ratio of the acid and alkaline standard colors is then varied. If the sample is turbid or colored, auxiliary cups are used according to the Walpole technique. A schematic diagram of a modified Duboseq colorimeter for this purpose is shown in Figure 105.

The procedure is also adapted to instrument <sup>8,9</sup>  
and is another form of the Gillespie Method.

**Procedure** <sup>10</sup>—In the right hand place concentration of indicator so selected that useful range. In the two cups on the left hand, of the indicator in the lower cup and the acid form in the upper. Adjust the cup containing standard and that containing the alkaline form of the indicator so that they are at the same definite distance from the plunger. Values of 10 mm., 15 mm. and 20 mm. are considered in the accompanying tables. Turn the , cup touches the plunger. By adjustment left hand cup depths of acid and alkaline color are of ant

When a match is obtained the pH

this the value of  $pK_a = \log \frac{1}{K_a}$ , the dissociation ex-  
constant. Having

the pK value,

pH =

As applied to the the  
—  $\alpha$  and the depth color  $\epsilon$

to

TABLE 32—

be obtained without calculation.

TABLE 32. READINGS FOR BALANCING TYPE COLORIMETER

Solution of the factor $\log \frac{y-x}{x}$ for 10 mm. depth											
y=10	x	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
1.0	0.95	0.91	0.86	0.82	0.79	0.75	0.72	0.69	0.66	0.63	
2.0	0.60	0.57	0.55	0.52	0.50	0.48	0.45	0.43	0.41	0.39	
3.0	0.37	0.35	0.33	0.31	0.29	0.27	0.25	0.23	0.21	0.19	
4.0	0.17	0.16	0.14	0.12	0.10	0.09	0.07	0.05	0.03	0.02	
5.0	0.00	0.98	0.96	0.95	0.93	0.91	0.89	0.88	0.86	0.84	
6.0	9.82	9.80	9.79	9.77	9.75	9.73	9.71	9.69	9.67	9.65	
7.0	9.63	9.61	9.59	9.57	9.55	9.52	9.50	9.48	9.45	9.42	
8.0	9.40	9.37	9.34	9.31	9.28	9.25	9.21	9.17	9.13	9.09	
9.0	9.05	8.91	8.87								

<sup>8</sup> V. C. Myers and L. E. Booher, *Proc. Soc. Exptl. Biol. Med.* 22, 511-2 (1924-5).

<sup>9</sup> J. McCrae, *Analyst* 51, 287-90 (

<sup>10</sup> J. J. Beaver, *J. Optical Soc. Am.* 18, 29).

N. Bjerrum, *Z. physik. Chem.* 104, 147 ; *Ibid.* 106, 219 (1923).

# READINGS FOR BALANCING TYPE COLORIMETER 705

TABLE 32. READINGS FOR BALANCING TYPE COLORIMETER—*Continued.*

Solution of the factor  $\log \frac{y-x}{x}$  for 15 mm. depth

$y=1$

x	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
1.0	1.15	1.10	1.06	1.02	0.99	0.95	0.92	0.89	0.86	0.84
2.0	0.81	0.79	0.76	0.74	0.72	0.70	0.68	0.66	0.64	0.62
3.0	0.60	0.58	0.57	0.55	0.53	0.52	0.50	0.48	0.47	0.45
4.0	0.44	0.42	0.41	0.40	0.38	0.37	0.35	0.34	0.33	0.31
5.0	0.30	0.29	0.28	0.26	0.25	0.24	0.22	0.21	0.20	0.19
6.0	0.18	0.16	0.15	0.14	0.13	0.12	0.10	0.09	0.08	0.07
7.0	0.06	0.05	0.03	0.02	0.01	0.00	0.99	0.98	0.97	0.95
8.0	9.94	9.93	9.92	9.91	9.90	9.88	9.87	9.86	9.85	9.84
9.0	9.82	9.81	9.80	9.79	9.78	9.76	9.75	9.74	9.72	9.71
10.0	9.70	9.69	9.67	9.66	9.65	9.63	9.62	9.60	9.59	9.58
11.0	9.56	9.55	9.53	9.52	9.50	9.48	9.47	9.45	9.43	9.42
12.0	9.40	9.38	9.36	9.34	9.32	9.30	9.28	9.26	9.24	9.21
13.0	9.19	9.16	9.14	9.13	9.08	9.05	9.01	8.98	8.94	8.90
14.0	8.85									
15.0										

Solution of the factor  $\log \frac{y-x}{x}$  for 20 mm. depth.

$y=20$

x	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
1.0	1.28	1.23	1.19	1.16	1.12	1.09	1.06	1.03	1.00	0.98
2.0	0.95	0.93	0.91	0.89	0.87	0.84	0.82	0.81	0.79	0.77
3.0	0.75	0.74	0.72	0.70	0.69	0.67	0.65	0.64	0.63	0.62
4.0	0.60	0.59	0.58	0.56	0.55	0.54	0.52	0.51	0.50	0.49
5.0	0.48	0.46	0.45	0.44	0.43	0.42	0.41	0.40	0.39	0.38
6.0	0.37	0.36	0.35	0.34	0.33	0.32	0.31	0.30	0.29	0.28
7.0	0.27	0.26	0.25	0.24	0.23	0.22	0.21	0.20	0.19	0.18
8.0	0.18	0.17	0.16	0.15	0.14	0.13	0.12	0.11	0.10	0.10
9.0	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.03	0.02	0.01
10.0	0.00	9.99	9.98	9.97	9.96	9.95	9.95	9.94	9.93	9.92
11.0	9.91	9.90	9.89	9.89	9.88	9.87	9.86	9.85	9.84	9.83
12.0	9.82	9.81	9.81	9.80	9.79	9.78	9.77	9.76	9.75	9.74
13.0	9.73	9.72	9.71	9.70	9.69	9.68	9.67	9.66	9.65	9.64
14.0	9.63	9.62	9.61	9.60	9.59	9.58	9.57	9.56	9.55	9.53
15.0	9.52	9.51	9.50	9.49	9.47	9.46	9.45	9.44	9.42	9.41
16.0	9.40	9.38	9.37	9.36	9.34	9.33	9.31	9.30	9.28	9.26
17.0	9.25	9.23	9.21	9.19	9.18	9.16	9.14	9.12	9.09	9.07
18.0	9.05	9.02	8.99	8.97	8.94	8.91	8.88	8.84	8.80	8.76
19.0	8.72	8.67	8.62	8.56	8.49	8.41	8.31	8.18	8.00	7.70



TABLE 33. READINGS FOR BALANCING TYPE COLORIMETER WHEN  
STANDARD COLUMN IS 15 MM.—*Continued*

Colori- meter Read- ings in mm.	pH m- Cresol Purple	pH Thymol Blue (acid)	pH Bromo- phenol Blue	pH Bromo- cresol Green	pH Chloro- phenol Red	pH Bromo- phenol Red	pH Bromo- cresol Purple	pH Bromo- thymol Blue	pH Phenol Red	pH m- Cresol Purple	pH Thymol Blue
11.0			3.54	4.23	5.54	5.72	5.86	6.56	7.46	7.88	8.46
11.2			3.51	4.20	5.51	5.69	5.83	6.53	7.43	7.85	8.43
11.4			3.48	4.17	5.48	5.66	5.80	6.50	7.40	7.82	8.40
11.6			3.45	4.14	5.45	5.63	5.77	6.47	7.37	7.79	8.37
11.8			3.41	4.10	5.41	5.59	5.73	6.43	7.33	7.75	8.33
12.0			3.38	4.07	5.38	5.56	5.70	6.40	7.30	7.72	8.30
12.2			3.34	4.03	5.34	5.52	5.66	6.36	7.26	7.68	8.26
12.4			3.30	3.99	5.30	5.48	5.62	6.32	7.22	7.64	8.22
12.6			3.26	3.95	5.26	5.44	5.58	6.28	7.18	7.60	8.18
12.8			3.22	3.91	5.22	5.40	5.54	6.24	7.14	7.56	8.14
13.0			3.17	3.86	5.17	5.35	5.49	6.19	7.09	7.51	8.09
13.2			3.12	3.81	5.12	5.30	5.44	6.14	7.04	7.46	8.04
13.4			3.06	3.75	5.06	5.24	5.38	6.08	6.98	7.40	7.98
13.6			2.99		4.99	5.17	5.31	6.01	6.91	7.33	
13.8					4.92	5.10	5.24	5.94	6.84		
14.0					4.83		5.15		6.75		

TABLE 34. READINGS FOR BALANCING TYPE COLORIMETER WHEN STANDARD COLUMN IS 10 MM.

Colori- meter Read- ings in mm.	pH m- Cresol Purple	pH Thymol Blue (acid)	pH Bromo- phenol Blue	pH Bromo- cresol Green	pH Chloro- phenol Red	pH Bromo- phenol Red	pH Bromo- cresol Purple	pH Bromo- thymol Blue	pH Phenol Red	pH m- Cresol Purple	pH Thymol Blue
1.0	2.46	2.45									
1.2	2.37	2.36									
1.4	2.30	2.29		5.46							
1.6	2.23	2.22		5.39						9.05	9.62
1.8	2.17	2.16	4.64	5.33						8.99	9.56
2.0	2.11	2.10	4.58	5.27				7.60		8.93	9.50
2.2	2.06	2.05	4.53	5.22	6.53			7.55		8.87	9.45
2.4	2.01	2.00	4.48	5.17	6.48		6.80	7.50	8.40	8.82	9.40
2.6	1.96	1.95	4.43	5.12	6.43		6.75	7.45	8.35	8.77	9.35
2.8	1.92	1.91	4.39	5.08	6.39		6.71	7.41	8.31	8.73	9.31
3.0	1.88	1.87	4.35	5.04	6.35		6.67	7.37	8.27	8.69	9.27
3.2	1.84	1.83	4.31	5.00	6.31		6.63	7.33	8.23	8.65	9.23
3.4	1.80	1.79	4.27	4.96	6.27		6.59	7.29	8.19	8.61	9.19
3.6	1.76	1.75	4.23	4.92	6.23	6.41	6.55	7.25	8.15	8.57	9.15
3.8	1.72	1.71	4.19	4.88	6.19	6.37	6.51	7.21	8.11	8.53	9.11
4.0	1.68	1.67	4.15	4.84	6.15	6.33	6.47	7.17	8.07	8.49	9.07
4.2	1.65	1.64	4.12	4.81	6.12	6.30	6.44	7.14	8.04	8.46	9.04
4.4	1.61	1.60	4.08	4.77	6.08	6.26	6.40	7.10	8.00	8.42	9.00
4.6	1.58	1.57	4.05	4.74	6.05	6.23	6.37	7.07	7.97	8.39	8.97
4.8	1.54	1.53	4.01	4.70	6.01	6.19	6.33	7.03	7.93	8.35	8.93
5.0	1.51	1.50	3.98	4.67	5.98	6.16	6.30	7.00	7.90	8.32	8.90
5.2	1.47	1.46	3.94	4.63	5.94	6.12	6.26	6.96	7.86	8.28	8.86
5.4	1.44	1.43	3.91	4.60	5.91	6.09	6.23	6.93	7.83	8.25	8.83
5.6	1.40	1.39	3.87	4.56	5.87	6.05	6.19	6.89	7.79	8.21	8.79
5.8	1.37	1.36	3.84	4.53	5.84	6.02	6.16	6.86	7.76	8.18	8.76
6.0	1.33	1.32	3.80	4.49	5.80	5.98	6.14	6.84	7.74	8.16	8.74
6.2	1.30	1.29	3.77	4.46	5.77	5.95	6.11	6.81	7.71	8.13	8.71
6.4	1.26	1.25	3.73	4.42	5.73	5.91	6.07	6.77	7.67	8.09	8.67
6.6	1.22	1.21	3.69	4.38	5.69	5.87	6.03	6.73	7.63	8.05	8.63
6.8	1.18	1.17	3.65	4.34	5.65	5.83	5.99	6.69	7.59	8.01	8.59
7.0	1.14	1.13	3.61	4.30	5.61	5.79	5.95	6.65	7.55	7.97	8.55
7.2	1.10	1.09	3.57	4.26	5.57	5.75	5.91	6.61	7.51	7.93	8.51
7.4	1.06	1.05	3.53	4.22	5.53	5.71	5.87	6.57	7.47	7.89	8.47
7.6			3.48	4.17	5.48	5.66	5.82	6.52	7.42	7.84	8.42
7.8			3.43	4.12	5.43	5.61	5.77	6.47	7.37	7.79	8.37
8.0			3.38	4.07	5.38	5.56	5.72	6.42	7.32	7.74	8.32
8.2			3.32	4.01	5.32	5.50	5.66	6.36	7.26	7.68	8.26
8.4			3.26	3.95	5.26	5.44	5.60	6.30	7.20	7.62	8.20
8.6			3.19	3.88	5.19	5.37	5.53	6.23	7.13	7.55	8.13
8.8			3.11	3.80	5.11	5.29	5.45	6.15	7.05	7.47	8.05
9.0			3.03		5.03	5.21	5.37	6.07	6.97	7.39	7.97

# READINGS FOR BALANCING TYPE COLORIMETER 709

TABLE 35. READINGS FOR BALANCING TYPE COLORIMETER WHEN STANDARD COLUMN IS 20 MM.

Colori- meter Read- ings in mm.	pH m- Cresol Purple	pH Thymol Blue (acid)	pH Bromo- phenol Blue	pH Bromo- cresol Green	pH Chloro- phenol Red	pH Bromo- phenol Red	pH Bromo- cresol Purple	pH Bromo- thymol Blue	pH Phenol Red	pH m- Cresol Purple	pH Thymol Blue
1.0	2.79	2.78									
1.2	2.70	2.69									
1.4	2.63	2.62									
1.6	2.57	2.56									
1.8	2.51	2.50									
2.0	2.46	2.45									
2.2	2.42	2.41									
2.4	2.38	2.37									
2.6	2.33	2.32									
2.8	2.30	2.29									
3.0	2.26	2.25									
3.2	2.23	2.22									9.62
3.4	2.20	2.19								9.01	9.59
3.6	2.16	2.15								8.97	9.55
3.8	2.14	2.13	4.61							8.95	9.53
4.0	2.11	2.10	4.58					7.60		8.92	9.50
4.2	2.09	2.08	4.56					7.58		8.90	9.48
4.4	2.06	2.06	4.53					7.55	8.45	8.87	9.45
4.6	2.03	2.03	4.50					7.52	8.42	8.84	9.42
4.8	2.01	2.00	4.48				6.80	7.50	8.40	8.82	9.40
5.0	1.99	1.98	4.46				6.78	7.48	8.38	8.80	9.38
5.2	1.96	1.95	4.43		6.43		6.75	7.45	8.35	8.77	9.35
5.4	1.94	1.93	4.41	5.10	6.41		6.73	7.42	8.32	8.75	9.32
5.6	1.92	1.91	4.39	5.08	6.39		6.71	7.41	8.31	8.73	9.31
5.8	1.90	1.89	4.37	5.06	6.37		6.69	7.39	8.29	8.71	9.29
6.0	1.88	1.87	4.35	5.04	6.35		6.67	7.37	8.27	8.69	9.27
6.2	1.86	1.85	4.33	5.02	6.33		6.65	7.35	8.25	8.67	9.25
6.4	1.84	1.83	4.31	5.00	6.31		6.63	7.33	8.23	8.65	9.23
6.6	1.82	1.81	4.29	4.98	6.29		6.61	7.31	8.21	8.63	9.21
6.8	1.80	1.79	4.27	4.96	6.27		6.59	7.29	8.19	8.61	9.19
7.0	1.78	1.77	4.25	4.94	6.25		6.57	7.27	8.17	8.59	9.17
7.2	1.76	1.75	4.23	4.92	6.23	6.41	6.55	7.25	8.15	8.57	9.15
7.4	1.74	1.73	4.21	4.90	6.21	6.39	6.53	7.23	8.13	8.55	9.13
7.6	1.72	1.71	4.19	4.88	6.19	6.37	6.51	7.21	8.11	8.53	9.11
7.8	1.70	1.69	4.17	4.86	6.17	6.35	6.49	7.19	8.09	8.51	9.09
8.0	1.69	1.68	4.16	4.85	6.16	6.34	6.48	7.18	8.08	8.50	9.08
8.2	1.67	1.66	4.14	4.83	6.14	6.32	6.46	7.16	8.06	8.48	9.06
8.4	1.65	1.64	4.12	4.81	6.12	6.30	6.44	7.14	8.04	8.46	9.04
8.6	1.63	1.62	4.10	4.79	6.10	6.28	6.42	7.12	8.02	8.44	9.02
8.8	1.61	1.60	4.08	4.77	6.08	6.26	6.40	7.10	8.00	8.42	9.00
9.0	1.60	1.59	4.06	4.76	6.07	6.25	6.39	7.09	7.99	8.41	8.99
9.2	1.58	1.57	4.05	4.74	6.05	6.23	6.37	7.07	7.97	8.39	8.97
9.4	1.56	1.55	4.03	4.72	6.03	6.21	6.35	7.05	7.95	8.37	8.95
9.6	1.54	1.53	4.01	4.70	6.01	6.19	6.33	7.03	7.93	8.35	8.93
9.8	1.53	1.52	4.00	4.69	6.00	6.18	6.32	7.02	7.92	8.34	8.92
10.0	1.51	1.50	3.98	4.67	5.98	6.16	6.30	7.00	7.90	8.32	8.90

TABLE 35. READINGS FOR BALANCING TYPE COLORIMETER WHEN STANDARD  
COLUMN IS 20 MM.—*Continued*

Colori- meter Read- ings in mm.	pH m- Cresol Purple	pH Thymol Blue (acid)	pH Bromo- phenol Blue	pH Bromo- cresol Green	pH Chloro- phenol Red	pH Bromo- phenol Red	pH Bromo- cresol Purple	pH Bromo- thymol Blue	pH Phenol Red	pH m- Cresol Purple	pH Thymol Blue
10.2	1.49	1.48	3.96	4.65	5.96	6.14	6.28	6.98	7.88	8.30	8.88
10.4	1.47	1.46	3.94	4.64	5.94	6.12	6.26	6.96	7.86	8.28	8.86
10.6	1.46	1.45	3.93	4.62	5.93	6.11	6.25	6.95	7.85	8.27	8.85
10.8	1.44	1.43	3.91	4.60	5.91	6.09	6.23	6.93	7.83	8.25	8.83
11.0	1.42	1.41	3.89	4.58	5.89	6.07	6.21	6.91	7.81	8.23	8.81
11.2	1.40	1.39	3.88	4.57	5.87	6.05	6.19	6.89	7.79	8.21	8.79
11.4	1.39	1.38	3.86	4.55	5.86	6.04	6.18	6.88	7.78	8.20	8.78
11.6	1.37	1.36	3.84	4.53	5.84	6.02	6.16	6.86	7.76	8.18	8.76
11.8	1.35	1.34	3.82	4.51	5.82	6.00	6.14	6.84	7.74	8.16	8.74
12.0	1.33	1.32	3.81	4.50	5.80	5.98	6.12	6.82	7.72	8.14	8.72
12.2	1.32	1.31	3.79	4.48	5.79	5.97	6.11	6.81	7.71	8.13	8.71
12.4	1.30	1.29	3.77	4.46	5.77	5.95	6.09	6.79	7.69	8.11	8.69
12.6	1.28	1.27	3.75	4.44	5.75	5.93	6.07	6.77	7.67	8.09	8.67
12.8	1.26	1.25	3.73	4.42	5.73	5.91	6.05	6.75	7.65	8.07	8.65
13.0	1.24	1.23	3.71	4.40	5.71	5.89	6.03	6.73	7.63	8.05	8.63
13.2	1.22	1.21	3.69	4.38	5.69	5.87	6.01	6.71	7.61	8.03	8.61
13.4	1.20	1.19	3.67	4.36	5.67	5.85	5.99	6.69	7.59	8.01	8.59
13.6	1.18	1.17	3.65	4.34	5.65	5.83	5.97	6.67	7.57	7.99	8.57
13.8			3.63	4.32	5.63	5.81	5.95	6.65	7.55	7.97	8.55
14.0			3.61	4.30	5.61	5.79	5.93	6.63	7.53	7.95	8.53
14.2			3.59	4.28	5.59	5.77	5.91	6.61	7.51	7.93	8.51
14.4			3.57	4.26	5.57	5.75	5.89	6.59	7.49	7.91	8.49
14.6			3.55	4.24	5.55	5.73	5.87	6.57	7.47	7.89	8.47
14.8			3.53	4.22	5.53	5.71	5.85	6.55	7.45	7.87	8.45
15.0			3.50	4.19	5.50	5.68	5.82	6.52	7.42	7.84	8.42
15.2			3.48	4.17	5.48	5.66	5.80	6.50	7.40	7.82	8.40
15.4			3.45	4.14	5.45	5.63	5.77	6.47	7.37	7.79	8.37
15.6			3.43	4.12	5.43	5.61	5.75	6.45	7.35	7.77	8.35
15.8			3.40	4.09	5.40	5.58	5.72	6.42	7.32	7.74	8.32
16.0			3.38	4.07	5.38	5.56	5.70	6.40	7.30	7.72	8.30
16.2			3.35	4.04	5.35	5.53	5.67	6.37	7.27	7.69	8.27
16.4			3.32	4.01	5.32	5.50	5.64	6.34	7.24	7.66	8.24
16.6			3.29	3.98	5.29	5.47	5.61	6.31	7.21	7.63	8.21
16.8			3.26	3.95	5.26	5.44	5.58	6.28	7.18	7.60	8.18
17.0			3.23	3.92	5.23	5.41	5.55	6.25	7.15	7.57	8.15
17.2			3.19	3.88	5.19	5.37	5.51	6.21	7.11	7.53	8.11
17.4			3.16	3.85	5.16	5.34	5.48	6.18	7.08	7.50	8.08
17.6			3.12	3.81	5.12	5.30	5.44	6.14	7.04	7.46	8.04
17.8			3.07	3.76	5.07	5.25	5.39	6.09	6.99	7.41	7.99
18.0			3.03	3.72	5.03	5.21	5.35	6.05	6.95	7.37	
18.2			2.97	3.66	4.97	5.15	5.29	5.99	6.89		
18.4					4.92		5.24		6.84		
18.6					4.86		5.18		6.78		
18.8					4.78						
19.0					4.70						



## PULFRICH PHOTOMETER

Read the solution in the photometer at 2 different wave lengths.<sup>11a</sup> Results should then be obtained from a curve for the ratio of the photometric readings. Table 36 gives the pH ranges and wave lengths for readings of the photometer with common indicators covering the normal range.

TABLE 36. WAVE LENGTHS FOR READING INDICATORS BY PULFRICH PHOTOMETER

Indicator	pH Range	Wave Lengths
Thymol blue .....	1.0-3.0	530-430
Bromophenol blue .....	3.0-4.4	430-570
Methyl red .....	4.4-6.27	530-430
Bromothymol blue .....	6.0-8.0	430-610
Thymol blue .....	8.0-10.0	430-570

## BLOOD, SERUM OR PLASMA

This is complicated by two factors. The value at 38° is that desired and the protein error may be large. The error due to temperature differences is the greater.

On 103 samples a correction of 0.22 pH to the colorimetric method gave agreement within 0.04 pH with the electrometric value at 38° in 85 per cent of the samples.<sup>12</sup> Similarly the correction for dog plasma is 0.30 pH. More recent work<sup>13</sup> on 45 samples indicates that a correction of 0.3 is nearly correct for humans.

Bromothymol blue has been reported as unsuitable for use with serum. A 0.02 per cent concentration may give an error as great as 0.8 pH.<sup>14</sup> Values for blood may be taken with *o*-chrom-T or *p*-nitrophenol.<sup>15</sup> The latter is a rather strong precipitating agent for proteins. Special cups with tight fitting covers must be used, the plasma introduced under the indicator solution, causing it to overflow, and the cover put in place.

Hähnel, *Svensk. Kem. Tids.* **46**, 262-79 (1934).

<sup>12</sup> Victor C. Myers and Edward Muntwyler, *J. Biol. Chem.* **78**, 243-55 (1928).

<sup>13</sup> Victor C. Myers, Edward Muntwyler, Dorothy Binns and Wayne H. Danielson, *J. Biol. Chem.* **102**, 19-28 (1933).

<sup>14</sup> D. Jaumain, *Compt. rend. soc. biol.* **93**, 860-2 (1925).

<sup>15</sup> J. F. McClendon, Sidney Russell and Edward Tracy, *J. Biol. Chem.* **70**, 705-11 (1926).

McClendon, Russell and Tracy criticize dichromatic indicators for use with blood. Cyanine cannot be used because it is affected by proteins.

A special procedure is required for the technique given because a special saline indicator is required. Satisfactory results have been reported.<sup>16</sup> The technique is approximately that of the Gillespie-Hatfield method. The standards are 0.01–0.02 pH more acid with the saline solution than in the absence of salt. The standards remain practically unchanged for 8 days. The colorimetric results agree within 0.02 pH with electrometric determination at the same temperature. The acid and alkaline solutions as used are according to Gillespie and obviate the necessity of phosphate buffers. The corpuscles do not interfere so that the results obtained are those of the plasma, even when whole blood is used.<sup>17</sup> Some error may be introduced by the 21-fold dilution but none was found experimentally on dilution.<sup>18</sup>

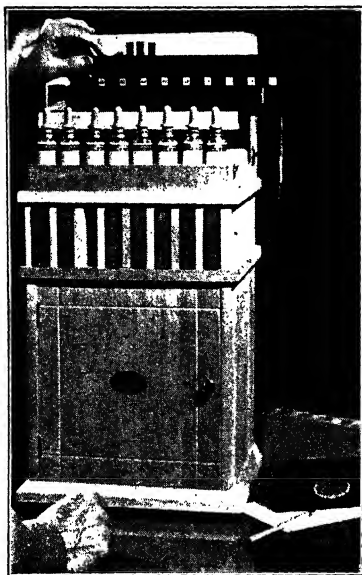


FIG. 107

Slide Comparator for pH Determination,  
With Built-in Illuminating System.  
(Courtesy of Holmes I. Mettee,  
Baltimore, Md.)

**Reagents** <sup>18</sup>—Dissolve 0.9 gram of sodium chloride in freshly redistilled water in a 100 cc. volumetric flask. Dilute 15 cc. of 0.1 per cent phenol red solution to 200 cc. to give a 0.0075 per cent solution. For use with whole blood add 11.0 cc. of this indicator solution to the saline solution and dilute to 100 cc. For use with plasma prepare a similar solution using 10.5 cc. of indicator solution. Prepare a similar solution by dilution of 11.0 cc. of distilled water to 100 cc. with the saline solution for use in

<sup>16</sup> Russell J. Fosbinder and Janetta W. Schoonover, *Biochem. J.* 24, 1805-10 (1930).

<sup>17</sup> G. E. Cullen, *J. Biol. Chem.* 52, 501-15 (1922).

A. B. Hastings and J. Sendroy, Jr., *J. Biol. Chem.* 61, 695-710 (1924).

the control tubes. Adjust the reaction of the saline indicators to approximately that of blood by covering the solution with mineral oil and adding 0.01 *N* sodium hydroxide solution containing the same concentration of indicator and salt,<sup>20</sup> drop by drop, until the pH is approximately 7.4. So adjusted there is little change on standing for a few hours. As standard acid use 0.0001 *N* hydrochloric acid and as alkali 0.01 *N* sodium hydroxide solution.

**Procedure** <sup>18</sup>—*Serum or Plasma*. Prepare the series of pH standards given in Table 37.

TABLE 37. TABLE OF pH VALUES AT 20° AND 38° AT 0.05 INTERVALS, WITH CORRESPONDING AMOUNTS OF 0.0075 PER CENT PHENOL RED AND 0.01 *N* NaOH OR 0.0001 *N* HCl.

pH 20°	Alkali tube		Acid tube		pH 38°	Alkali tube		Acid tube	
	<i>cc. dye</i>	<i>cc. alkali</i>	<i>cc. dye</i>	<i>cc. acid</i>		<i>cc. dye</i>	<i>cc. alkali</i>	<i>cc. dye</i>	<i>cc. acid</i>
6.70	0.19	24.81	2.31	22.69	6.70	0.25	24.75	2.25	22.75
6.75	0.21	24.79	2.29	22.71	6.75	0.28	24.72	2.22	22.78
6.80	0.24	24.76	2.26	22.74	6.80	0.31	24.69	2.19	22.81
6.85	0.26	24.74	2.24	22.76	6.85	0.34	24.66	2.16	22.84
6.90	0.29	24.71	2.21	22.79	6.90	0.38	24.62	2.12	22.88
6.95	0.32	24.68	2.18	22.82	6.95	0.42	24.58	2.08	22.92
7.00	0.36	24.64	2.14	22.86	7.00	0.46	24.54	2.04	22.96
7.05	0.39	24.61	2.11	22.89	7.05	0.50	24.50	2.00	23.00
7.10	0.43	24.57	2.07	22.93	7.10	0.55	24.45	1.95	23.05
7.15	0.48	24.52	2.02	22.98	7.15	0.60	24.40	1.90	23.10
7.20	0.52	24.48	1.98	23.02	7.20	0.65	24.35	1.85	23.15
7.25	0.57	24.43	1.93	23.07	7.25	0.71	24.29	1.79	23.21
7.30	0.62	24.38	1.88	23.12	7.30	0.77	24.23	1.73	23.27
7.35	0.68	24.32	1.82	23.18	7.35	0.84	24.16	1.66	23.34
7.40	0.74	24.26	1.76	23.24	7.40	0.90	24.10	1.60	23.40
7.45	0.80	24.20	1.70	23.30	7.45	0.97	24.03	1.53	23.47
7.50	0.86	24.14	1.64	23.36	7.50	1.04	23.96	1.46	23.54
7.55	0.93	24.07	1.57	23.43	7.55	1.11	23.89	1.39	23.61
7.60	1.00	24.00	1.50	23.50	7.60	1.18	23.82	1.32	23.68
7.65	1.07	23.93	1.43	23.57	7.65	1.25	23.75	1.25	23.75
7.70	1.14	23.86	1.36	23.64	7.70	1.32	23.68	1.18	23.82
7.75	1.21	23.79	1.29	23.71	7.75	1.39	23.61	1.11	23.89
7.80	1.28	23.72	1.22	23.78	7.80	1.46	23.54	1.04	23.96
7.85	1.35	23.65	1.15	23.85	7.85	1.53	23.47	0.97	24.03
7.90	1.42	23.58	1.08	23.92	7.90	1.60	23.40	0.90	24.10
7.95	1.49	23.51	1.01	23.99	7.95	1.67	23.33	0.83	24.17
8.00	1.56	23.44	0.94	24.06	8.00	1.73	23.27	0.77	24.23

<sup>20</sup> Janetta W. Schoonover and Gladys E. Woodward, *J. Lab. Clin. Med.* 16, 621-4 (1931).

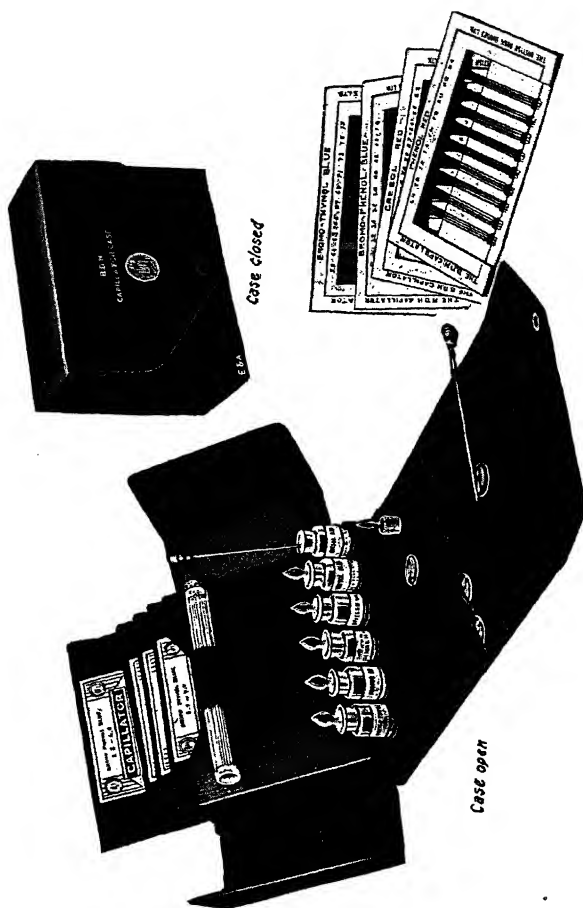


Fig. 108

British Drug House Capillary Colorimeter. (Courtesy of Eimer & Amend)

Put 4 cc. of adjusted saline indicator in a test tube under oil. Add 0.2 cc. of blood serum to the indicator and stir. Prepare a control using the sample and saline solution without indicator. Replace the oil by paraffin and place a thermometer in the cork with the bulb dipping into the control or a tube of water.

Prepare tubes containing 5 cc. portions of the standards to be used at the same time, similarly protected by paraffin. Heat to 38.5 to 39° and when the temperature has dropped to 38° make the comparison promptly, using the technique of the Gillespie-Hatfield method for colored samples. The standards may be heated or not according to the series used. The standards may be kept for 5 weeks without fading.<sup>20</sup>

*Blood.* Follow the above procedure except that the indicator of a different concentration for blood is used. After mixing, centrifuge to settle the corpuscles before proceeding.

### COLORED SOLUTIONS

The estimation of pH in colored solutions by the Walpole technique requires one additional step. Buffer solutions, the Gillespie-Hatfield method or the balancing method may be used. The procedure is applicable only if the color is not too intense and the turbidity slight.

**Procedure Using Buffers**—Treat 10 cc. of sample in 1 tube by the usual procedure. In another tube place 10 cc. of untreated sample.

For estimation of the pH compare the total color of the sample tube and one or more tubes of distilled water with the total color of the standard or standards and the untreated sample. In every case therefore the sum of 2 or of 3 tubes is compared.

**Procedure without Buffers**—By the procedure 2 tubes are usually compared with 2 others. For colored solutions 3 are compared, the treated sample and 2 tubes of distilled water with the 2 standard tubes and 1 tube of untreated sample.

**Balancing Method**—Auxiliary cups of fixed depth are used below the cups previously described. In the right hand auxiliary cup place the unknown with the indicator, instead of in the next cup above. In the left hand auxiliary cup place the unknown without the indicator. Fill

depth  
 a layer of fixed depth of sample  
 through an equal depth of acid and alkaline  
 water. On the right the light passes through the sample  
 developed with an indicator, then through the same depth of distilled  
 water. From that point on the manipulation is the same as for a colorless  
 sample.

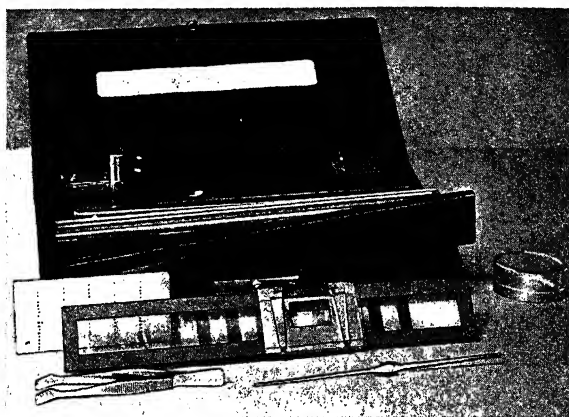


FIG. 109

Wulff Colorimeter for pH Determination. (Courtesy of Pfaltz & Bauer)

**Extrapolation** <sup>23</sup>—In order to determine the pH of a colored solution, dilute to 2, 4, 6, 8, etc. times the original volumes. Determine the pH of each according to one of the usual procedures. Plot the pH found against the logarithm of the dilution. The points should fall on a straight line and when extrapolated to zero dilution will give the pH of the original solution. Data with tan liquors check the electrometric method within about 0.05 pH unit.

<sup>23</sup> F. C. Thompson and W. R. Atkins, *J. Intern. Soc. Leather Trades Chem.* 13, 297-9 (1929).

## COMPARISON WITH TEST STRIPS

For rough approximations the colors of test papers moistened with various pH indicators are used. These methods are neither well developed nor accurate. A portable set of indicators for comparison with glass standards is on the market and claimed to be accurate to 0.1 pH.

A special paper has been used for milk.<sup>24</sup> To prepare this wash filter paper until neutral. Saturate with a 0.1 per cent solution of methyl red prepared according to the usual method. The paper is yellow. Fresh milk does not change the color but as the milk becomes more acid the color obtained is orange or orange-red.

A series of mixed indicators used on test papers show a neutral grey, varied to a color in each direction from the standard value.<sup>25</sup> Instead of paper, thin films of colorless cellulose acetate carrying the indicator are also used.<sup>26</sup> The pH of those colored solutions which do not stain the strips and of turbid solutions can be estimated by dipping for 1 to 2 minutes, rinsing and comparing with a scale. The indicator is not readily leached out. The entire range is not equally accurate. It failed with fertilizer extracts.<sup>27</sup>

## COMPARISON ON A SPOT PLATE

Another approximate method is by comparison of single drops on a vaselined plate<sup>28</sup> or of a few drops in a depression in a porcelain plate. The method, comparing with a chart of colors, is used for soil acidity.<sup>29</sup>

<sup>24</sup> A. Tapernoux, *Compt. rend. soc. biol.* **93**, 621-2 (1928).

<sup>25</sup> W. U. Behrens, *Z. anal. Chem.* **73**, 129-37 (1928).

<sup>26</sup> Peter Wulff, *Kolloid-Z.* **40**, 341-2 (1926).

<sup>27</sup> Erik Larsson, *Svensk. Kem. Tids.* **43**, 322-30 (1931).

<sup>28</sup> Oscar W. Richards, *Science* **68**, 185 (1928).

<sup>29</sup> M. F. Morgan, *Ecology* **8**, 387-8 (1927).





# AUTHOR INDEX

- Acree, S. F., 682, 694  
 Adams, E. T., 468, 469  
 Adams, G. O., 559  
 Adams, J., 432  
 Adler, L., 63  
 Adolph, W. H., 571  
 Agnew, W. J., 301, 326  
 Agulhon, H., 527  
 Ahearn, J. F., 70  
 Alekseeva, M. V., 611  
 Alekseevskii, E. V., 179  
 Alfthan, K., 542  
 Alimarin, I. P., 517  
 Allen, N., 139, 141  
 Allen, W. S., 226  
 Allison, V. C., 64, 632  
 Allott, E. N., 551  
 Allport, N. L., 168, 170, 187, 188  
 Almquist, H. J., 558, 562  
 Almy, L. H., 593, 595  
 Alten, F., 267, 271, 300, 339, 436, 439,  
 466, 474, 501, 655  
 Amati, A., 249  
 Ambler, J. A., 493, 521, 523  
 American Public Health Association, 288,  
 305, 336, 341, 538, 634, 642  
 American Society for Testing Materials,  
 85  
 Andreasch, R., 298  
 Andrews, L., 297  
 Andrews, R. L., 196  
 Andrisk, V., 112  
 Angel & Co., H. R., 57  
 Angelescu, E., 569  
 Ansbacher, S., 153, 154, 160, 164  
 Antipov-Karataev, I. N., 438  
 Aoyama, S., 534, 613  
 Archbutt, L., 238  
 Ardagh, E. G. R., 418, 419, 420  
 Armit, H. W., 315  
 Armstrong, W. D., 576, 577  
 Arnfeld, 517  
 Army, H. V., 66, 68, 79, 256, 657  
 Arrhenius, O., 492  
 Association of Official Agricultural Chem-  
 ists, 225, 232  
 Astrug, A., 463  
 Atack, F. W., 259, 323  
 Aten, A. H. W., 58  
 Atkins, W. R., 716  
 Atkins, W. R. G., 244, 500, 509, 517  
 Aubrey, P., 221  
 Austin, J. H., 669  
 Autenrieth, W., 55, 291, 559, 608  
 Baden, M. W., 57  
 Bader, J. P., 21  
 Badollet, M. S., 22  
 Baganz, G., 64  
 Bagchi, K. N., 551  
 Bailey, C. H., 693  
 Bailey, H. S., 21  
 Bailey, W. F., 432  
 Baizzo, R., 159  
 Baker, J. C., 30, 689  
 Balch, R. T., 96  
 Balconi, M., 389  
 Baldeschweiler, E. L., 208  
 Balfe, M. P., 151, 152, 294  
 Ball, A. A., 83  
 Ball, W. C., 444  
 Bamford, F., 252  
 Bang, I., 227  
 Banks, H. W., 91  
 Barber, H. H., 439, 440  
 Barkan, G., 283  
 Barker, P., 480, 520  
 Barneby, O. L., 359, 360  
 Barnes, H. D., 476  
 Barnes, J. W., 230  
 Barnett, C. W., 53, 55  
 Barnett, G. D., 53, 55  
 Barr, G., 581  
 Barrenscheen, H. K., 439, 442

- Barron, E. A. G., 298  
Barrows, W. P., 182  
Barton, L. E., 653  
Barton, P. D., 30  
Bartow, E., 332  
Baubigny, H., 548  
Baudouin, A., 51  
Bauer, E., 517, 518, 519  
Baumann, E. J., 488, 489, 497  
Baumann, J., 695  
Baus, R., 15  
Baver, L. D., 689  
Bawtree, A. E., 63, 94  
Baylis, J. R., 95  
Beale, 519  
Beamish, F. E., 229  
Beans, H. T., 280  
Bear, F. E., 630  
Beater, B. E., 493, 500  
Beaver, H. J., 689  
Beaver, J. J., 704  
Bečka, J., 476  
Becker, 53  
Beer, 3  
Beery, E. H., 148  
Behrens, W. U., 717  
Belcke, E., 269  
Belin, J., 243  
Bell, R. D., 444, 468, 497, 501, 521, 652  
Belladen, L., 241, 512  
Bellis, B., 152  
Bénard, H., 51, 459  
Bender, M., 333  
Bendig, M., 284, 356  
Benedict, S. R., 49, 505  
Bennett, M. A., 669  
Benoist, L., 142  
Benoit, C., 160  
Berg, R., 460, 472, 525, 604  
Berg, W., 57  
Berger, C., 64, 642  
Berger, L. B., 110  
Berggren, R. E. L., 506  
Bergheimer, E., 548  
Berl, E., 126  
Berman, L., 292, 298  
Bernhard, A., 283  
Bernheim, G., 26  
Bernouilli, A. L., 56, 309  
Bernstein, A., 62  
Bertrand, E., 391  
Bertrand, G., 332, 337, 523, 527  
Bickley, E. H., 65  
Biehler, W., 695  
Bier, A. 548  
Bülmann, E., 430  
Bilham, P., 153  
Bing, F. C., 64  
Binns, D., 711  
Birekner, V., 348  
Bird, 226  
Bird, O. D., 261, 262, 264, 498  
Bishop, W. B. S., 206  
Bjerrum, N., 704  
Black, O. F., 236  
Blair, A. A., 102, 103, 276, 332, 338, 359  
Blair, H., 440  
Blake, S. A., 87  
Blanchetière, A., 330, 435, 440  
Blass, J., 188  
Blau, F., 310  
Blodgett, G., 230  
Bloor, W. R., 91, 497, 498, 510, 511  
Bock, J. C., 49  
Bogen, E., 685  
Bogitch, F., 70  
Bogitch, L., 103  
Böhm, J. S., 384  
Bohn, H., 695  
Bohn, R. T., 693  
Boinot, G., 498, 612  
Bolshakov, K., 373  
Bond, J. D., 117, 119  
Bondzynski, S., 298  
Booge, J. E., 94  
Booher, L. E., 704  
Booth, H. S., 112, 115  
Booth, J. H. W., 47  
Boratyuski, K., 494  
Boruff, C. S., 539  
Bose, A. C., 551  
Bosworth, A. W., 497  
Bouisson, N., 461  
Bourcart, P., 22  
Boutwell, P. W., 95, 611, 612  
Boyd, J. I., 497

- Boynton, P., 687  
 Braley, S. A., 327  
 Brasi, M., 500  
 Braun, A. D., 384  
 Bray, W. C., 208, 233  
 Breed, R. S., 689  
 Bremner, R. W., 289  
 Breteau, P., 198  
 Brice, Jr., A. T., 62  
 Briggs, A. P., 454, 468, 501, 521  
 Bright, H. A., 346  
 Brinton, P. H. M.-P., 385  
 British Pharmacopoeia, 226  
 Britton, J. B., 70  
 Brode, W. R., 662  
 Brodigan, C., 404  
 Brotherhood, J. S., 15  
 Brown, B. E., 483  
 Brown, F. E., 246  
 Brown, J. H., 690  
 Brück, E., 187, 346  
 Bruère, P., 71, 334  
 Brukner, 53  
 Bruttini, A., 395  
 Bryant, E. C., 26  
 Buch, K., 510  
 Buchan, J. L., 191  
 Buchanan, J. H., 209  
 Budkov, N. I., 357  
 Burgess, W. T., 133  
 Burker, K., 64  
 Burmester, B. R., 298  
 Burton, D., 688  
 Burton, J. I., 382  
 Busby, H. S., 51  
 Butler, A. M., 440, 441  
 Byall, S., 493, 521, 523  
 Byers, H. G., 233, 234, 235, 501, 606  
  
 Cabanes, E., 47  
 Caille, M., 43  
 Cain, J. R., 284, 295, 297  
 Cake, W. E., 498  
 Calatroni, R., 533  
 Caley, E. R., 437, 439, 440, 447  
 Calfee, R. K., 554  
 Callan, T., 164, 165, 227, 603  
 Cameron, F. K., 431  
  
 Campbell, E. D., 39  
 Campbell, M. B., 112, 115  
 Canals, E., 47, 58  
 Cannon, R. K., 298  
 Capen, R. G., 333  
 Carey, F. P., 230  
 Carleton, P. W., 409, 532  
 Carpentier, G., 176  
 Carpiaux, E., 452  
 Carr, C. J., 96  
 Carter, R. H., 630  
 Casares, J., 579  
 Case, L. C., 565  
 Cassal, C. E., 526  
 Cassel, H. R., 402, 403  
 Cassoni, B., 283, 577  
 Castiglioni, A., 151, 603  
 Castille, A., 552  
 Cavazzi, A., 356  
 Cayvan, L. L., 365, 373, 480, 519, 520  
 Cazeneuve, A., 198, 279  
 Cazeneuve, M. P., 180  
 Cerdan, A. del C., 345  
 Chakmakjian, H. H., 304  
 Chalk, L. J., 160  
 Chambers, J. C., 163  
 Chamot, E. M., 629, 633, 634  
 Chandler, L. R., 689  
 Chapin, R. M., 542  
 Chapman, A. C., 129  
 Chapman, H. D., 503, 508  
 Cheftel, H., 188  
 Chevalier, R., 302  
 Chiles, H. M., 649, 652  
 Chouchak, D., 242, 511  
 Ciocalteu, V., 183  
 Claesson, P., 298  
 Claman, S., 96  
 Clark, H. W., 559  
 Clark, N. A., 333, 344  
 Clark, O. E., 668  
 Clark, R. J., 691  
 Clark, S. G., 163  
 Clark, W. M., 31, 88, 660, 671, 673, 677,  
 678, 681  
 Clarke, H. T., 612  
 Clarke, S. G., 189, 253

- Clarke, W. F., 225  
 Clausmann, P., 584  
 Clennell, J. E., 336  
 Clifford, S. G., 227  
 Coates, C. E., 93  
 Coch, G., 669  
 Cohen, B., 669, 681  
 Cohen, H. R., 456, 457, 500, 506  
 Collins, 226  
 Collins, H. L., 229  
 Collins, W. D., 13, 73, 335, 341, 346, 465, 618  
 Combes, A., 303  
 Comrie, A. D., 226, 232  
 Conn, L. W., 153, 160, 164  
 Connell, W. B., 125  
 Conover, C., 671  
 Cooke, J. H., 89, 285, 287  
 Cooksley, T., 206  
 Cooledge, L. H., 15  
 Cooper, L. H., 544, 566  
 Cooper, L. H. N., 486, 651  
 Corey, R. B., 261  
 Coulson, E. J., 225, 235, 236  
 Cousen, A., 606  
 Cox, G. J., 262  
 Crawford, W. G., 363, 364  
 Cray, F. W., 688  
 Crema, C., 511  
 Cribb, C. H., 226  
 Cribier, J., 238  
 Croner, F., 333  
 Crookes, W., 66  
 Crosen, R. G., 280  
 Crum, W., 332  
 Crumpler, T. B., 15, 58  
 Cuboni, E., 57  
 Cullen, G. E., 669, 712  
 Culp, F. B., 153, 154, 160, 164, 556  
 Cunningham, F. W., 57  
 Cunningham, I. J., 468  
 Cunningham, T. R., 384, 385, 387  
 Cuny, L., 221  
 Currie, A. N., 174  
 Cuvelier, B. V. J., 431  
 Cuvelier, V., 173  
 Czerny, R., 65, 288  
 Dahle, D., 571, 574  
 Daigo, T., 534, 613  
 Danekwortt, P. W., 196, 410  
 Danet, R., 645, 656  
 Daniel, H. A., 294  
 Danielson, W. H., 711  
 Das-Gupta, P. N., 365, 394  
 Daugherty, J. E., 70  
 Dauphinee, J. A., 551  
 David, M., 175  
 Davidson, J., 333  
 Davies, D. R., 494, 502  
 Davies, W. C., 494, 502  
 Davindov, A. L., 58  
 Davis, C. E., 682  
 Davis, C. W., 43, 413, 631, 633  
 Davis, G. H., 226, 227  
 Dawihl, W., 573  
 de Beus, J., 194  
 de Brouckère, L., 151, 269  
 Deckert, W., 351  
 Deemer, R. B., 225, 245, 501  
 Defay, R., 62  
 de Haan, J., 18  
 Dehn, W. M., 74, 75, 83, 274, 276  
 de la Puente, J., 345  
 Delaville, M., 243, 435  
 de Loureiro, J. A., 96, 511  
 Delrue, G., 222  
 Delpy, M., 126  
 Demkina, L. I., 65  
 de Nardo, L. U., 641  
 Deniges, G., 140, 156, 307, 310, 319, 434, 490, 500, 636  
 Denis, W., 468, 618  
 Denison, R. B., 65, 104, 120  
 Dérivéré, M., 12  
 Desbleck, L. B., 57  
 Desbleds, L. B., 86  
 Desch, C. H., 304  
 de Smet, P., 57  
 Desy, G., 602  
 De Turk, E. E., 496  
 Dhar, N. R., 569  
 Dibdin, W. J., 544, 566  
 Dieckmann, T., 358  
 Diehl, R., 677  
 Dienert, F., 517, 519

- Digand, C., 57  
 Dingwall, A., 280  
 di Nola, E., 406  
 Dittrich, M., 335  
 Dodd, A. S., 226  
 Dodge, C. W., 65  
 Dodge, F. D., 671, 672, 673  
 Doerner, H. A., 385  
 Doisy, E. A., 444, 468, 497, 501, 521  
 Dolinek, A., 57  
 Dominici, G., 284, 293  
 Donan, J., 51  
 Dopter, P., 688  
 Douglas, W. F., 669  
 Downs, H. R., 569  
 Drabkin, D. L., 153  
 Drake, N. L., 682  
 Dreguss, M., 433, 435  
 Dreyspring, C., 501  
 Dreker, I. J., 283  
 Drews, B., 25  
 Drillhon, A., 305  
 Drilhon, M., 305  
 Drushel, W. A., 598  
 Dubief, J., 565  
 Dudley, H. C., 233, 234, 235, 501, 606  
 Duez, P., 356, 367  
 Dufty, L., 334, 339  
 Duggar, B. M., 65  
 Dunajew, A., 494  
 Dunncliff, H. B., 156, 157  
 Dunnington, F. P., 356, 362  
 Duprey, M., 535, 536  
 Durgin, C. B., 190, 230, 536, 531, 672  
 Durupt, A., 437  
 Dutcher, H. A., 583  
 Duyk, M., 333, 340  
 Earle, I. P., 669  
 Eastlack, H. E., 94  
 Eckstein, H., 70  
 Eden, W., 695  
 Efimoff, W. W., 137  
 Egerer, G., 92, 241, 511  
 Eggertz, 70  
 Egorov, M. S., 142  
 Eichholtz, F., 460, 472  
 Elliot, F. A., 94  
 Ellis, N. R., 298  
 Ellims, J. W., 538  
 Elmer, A. W., 559  
 Elston, C. M., 598  
 Elvehjem, C. A., 160, 161, 290  
 Emerson, P., 631  
 Emmerie, A., 172  
 Emmert, E. M., 104, 121, 432, 455, 632  
 Enrich, F., 51  
 Enslow, L. H., 539, 542  
 Ericsson, A., 226  
 Erkkila, S., 690  
 Estes, C., 481, 514  
 Etzel, G., 152  
 Evans, B. S., 189, 197, 198, 251, 276, 279,  
     324  
 Evers, N., 164, 165  
 Ewald, W., 93  
 Exton, W. G., 64, 93  
 Faber, H., 433  
 Faber, J. E., 508  
 Faber, P., 356  
 Faillebin, M., 345  
 Failyer, G. H., 431, 463, 611, 634  
 Fairhall, L. T., 176, 314, 317, 318, 348  
 Falciola, P., 392  
 Falk, K. G., 70, 81  
 Farber, J. E., 245  
 Farr, H. V., 73  
 Fassin, G., 49  
 Fawcett, E. H., 682, 694  
 Fawsett, G. S., 27  
 Fedorova, O. S., 590, 591  
 Fegler, J., 117  
 Feher, D., 501  
 Feigl, F., 180, 310, 314, 343, 377, 453,  
     475, 500  
 Feinberg, S., 204  
 Felix, K. S., 57, 58  
 Fellenberg, T. von, 62, 551, 569  
 Fenner, G., 370  
 Ferguson, W. C., 218  
 Ferner, G. W., 670  
 Ferre, L., 294, 304  
 Ferrey, G. J. W., 642  
 Ferris, W. S., 470, 483  
 Fer'yanchich, F. S., 377

- Ficklen, J. B., 199, 258, 343, 474, 541  
 Fink, D. E., 239  
 Firth, J. B., 569  
 Fischer, H., 168, 202, 399  
 Fiske, C. H., 488, 497, 504, 505, 506, 621  
 Fleischer, I. F., 693  
 Fleming, R., 157  
 Fleury, P., 79, 198  
 Flinn, F. B., 244  
 Flint, C. F., 642  
 Florentin, D., 509  
 Fogel'son, E., 277  
 Foix, A., 406  
 Folin, O., 183, 305, 461, 505, 534, 652, 654  
 Folkard, C. W., 79  
 Fonteyne, R., 57  
 Forman, L., 341  
 Forrest, J. W., 27, 47  
 Forsberg, O., 538  
 Forster, F., 438  
 Fort, I., 492  
 Fosbinder, R. J., 712  
 Foster, M. D., 335, 341, 465, 529, 574, 618  
 Foulger, J. H., 523  
 Foulk, C. W., 439, 440  
 Fowweather, F. S., 283, 290, 291, 292, 293  
 Foxwell, G. E., 658  
 Francis, A. G., 191  
 Francis, E. K., 125  
 Franklin, M. C., 468  
 Frankforter, G. B., 130, 132  
 Fraps, G. S., 284, 304, 629, 633  
 Frederick, R. C., 39, 654  
 Freudenberg, E., 501  
 Freund, H., 64  
 Frick, C., 79  
 Fridli, R., 209  
 Frieden, A., 88  
 Friendenthal, H., 290  
 Friendländer, V., 280  
 Frost, E. C., 212, 213  
 Fry, J. S., 93  
 Fuchs, H. J., 655  
 Fudge, J. F., 284, 304, 500, 682  
 Fuld, E., 48  
 Fuller, C. H. F., 153  
 Fulton, C. C., 172  
 Funck, A. D., 380  
 Funk, A., 55, 559, 608  
 Funk, V. G., 334  
 Furman, H. H., 409  
 Gale, N., 128  
 Galema, N., 58  
 Galletti, A. C., 690  
 Gantois, 358  
 Gardner, H. A., 94  
 Gardner, W. M., 182  
 Garmash, E. P., 464  
 Garn, W. von, 604  
 Garratt, F., 278  
 Garreau, Y., 79  
 Gathercoal, E. N., 31  
 Gautier, A., 69, 356, 362, 584  
 Geagley, W. C., 569  
 Gebhardt, H. T., 160  
 Geilmann, W., 392  
 Georgescu, I., 161  
 Gerb, L., 193, 517, 518, 519  
 Gerdel, R. W., 430, 436  
 Gericke, W. F., 631  
 Germuth, F. G., 355, 645  
 Gerrans, H., 526  
 Gerritz, H. W., 450, 490, 493, 498, 501  
 Gerschmann, R., 437  
 Gerstacker, L., 96  
 Getz, C. A., 498  
 Gherardini, G., 500  
 Giani, M., 510  
 Gibbs, H. D., 671  
 Gibson, K. S., 32, 33, 34, 72  
 Gibson, R. B., 514  
 Gieger, M., 164  
 Giesecking, J. E., 498  
 Gilchrist, R., 416  
 Gill, L. M., 21  
 Gillespie, L. J., 12, 47, 697  
 Gimingham, C. T., 572  
 Gimington, C. T., 630  
 Ginsberg, H., 5, 355, 356  
 Ginsberg, I., 96  
 Ginsburg, J., 298  
 Ginsburg, J. M., 232  
 Givens, J. W., 558, 562

- Glassman, B., 212, 217  
Glick, D., 499  
Goebel, E., 693  
Goethals, C. A., 58  
Gol'denberg, Y. D., 280  
Gollnow, G., 689  
Golnow, G., 17, 58  
Golse, G., 185  
Golse, J., 161, 173  
Gomberg, M., 42  
Gooch, F. A., 447, 526, 527  
Gorbatscheff, S. W., 569  
Gordon, C. S., 110  
Gortner, R. A., 332, 335, 341  
Goulston, D., 687  
Gow, P. L., 430  
Goy, S., 689  
Gnaedinger, R. F., 94  
Gramenitski, M. J., 569  
Grant, J., 47  
Grant, N. S., 418, 419, 420  
Grau, E., 548  
Graves, S. S., 91, 657  
Greathouse, L. H., 332, 339  
Greaves, J. E., 629  
Green, E. I., 236  
Greene, C. H., 410, 689  
Greenwald, I., 497  
Gregoire, A., 449, 452, 478, 514  
Gregory, A. W., 301, 371  
Gregory, V. H., 56  
Grendel, F., 164, 165  
Griess, J. P., 646, 647  
Griffin, R. C., 236  
Gripenberg, S., 501  
Gross, C. R., 225, 227, 231, 236  
Grutzner, P. v., 55  
Guarnieri, P., 626  
Guelke, R., 62  
Guerrant, N. B., 489  
Guild, J., 27, 78, 79  
Guillaume, J., 686  
Guldina, E., 395  
Gunther, T., 39  
Guntz, A. A., 26, 51  
Gurevich, V. G., 601, 627  
Gustafson, F., 199  
Gustafson, F. G., 689  
Gutman, M., 490  
Gutzeit, G., 258  
Guyer, A., 501  
Guyot, R., 243  
Haase, L. W., 635  
Hackmann, C., 62  
Haddock, L. A., 164, 165  
Haensch, 93  
Hagues, G., 65  
Hahn, F., 548  
Hahn, F. F., 79  
Hähnel, S., 711  
Hale, F. E., 538, 540  
Hall, D., 383  
Hall, M., 432  
Hall, W. T., 274, 653, 654  
Hamburg, H., 310  
Hamburger, H. J., 434  
Hamence, J. H., 192  
Hamilton, J., 22  
Hamilton, Jr., R. H., 84  
Hammerster, O., 120  
Hammett, F. S., 51, 468, 469  
Hammett, L. P., 260  
Hamner, H. L., 384, 385, 387  
Hampshire, P., 691  
Hanak, A., 144, 149, 294, 304  
Hand, P. G. T., 133  
Hann, R. M., 262  
Hannan, F., 687  
Hanson, N., 33, 275, 276  
Hanssen, R., 257  
Hantzsch, A., 304  
Hanzel, R. F., 298, 299  
Harden, A., 315  
Harden, W. C., 682  
Hardy, A. C., 57, 65  
Harper, H. J., 294, 629, 630, 633  
Harris, F. K., 34  
Harrison, A. P., 87, 636  
Harry, R. G., 171, 342  
Hart, E. B., 290  
Hartman, R. J., 693  
Hartnagel, J., 494, 501  
Hartwell, B. L., 497  
Harvey, C. O., 191  
Harvey, T. F., 226

- Haskins, H. D., 71  
 Hastings, A. B., 712  
 Hatfield, W. D., 266, 698  
 Haupt, G. W., 32  
 Hauptmann, H., 389  
 Haury, V. G., 476  
 Hauser, S. J., 538  
 Hawk, B. W., 496  
 Hawk, P. B., 92  
 Hazen, A., 66  
 Health, B. V., 361  
 Heath, F. H., 593  
 Heath, R. F., 147, 377  
 Heck, A. F., 226  
 Hedin, S. G., 120  
 Heidenhain, H., 227, 230, 237  
 Heidelberg, T. v., 410, 533  
 Heinds, J. I. D., 611  
 Heinrichs, H., 222, 286  
 Heinz, W., 321, 323, 328, 330, 501  
 Heise, K., 283  
 Heller, H., 96  
 Hellige, Inc., 30  
 Hellige, P. A. E., 23  
 Helmrich, 58  
 Hemphill, G., 624, 625  
 Henderson, J. A. R., 164, 165, 603  
 Hennel, W., 515  
 Henri, V., 552  
 Herapath, T. J., 296  
 Hertrich, M., 222, 286  
 Hertwig, R., 147  
 Hertzog, E. S., 237, 238  
 Heslinga, J., 333, 340  
 Hessel, G., 176  
 Hewitt, T. E., 484  
 Heyne, G., 378, 379  
 Hibbard, P. L., 42, 492  
 Higgins, H. L., 117  
 Hijman, A. J., 203  
 Hill, 226  
 Hill, C. A., 215, 217  
 Hill, R., 310, 311  
 Hill, W. L., 259, 265  
 Hilla, L. A., 430  
 Hille, E., 271, 300, 655  
 Hillebrand, W. F., 275, 355, 356, 357, 497  
 Hiller, A., 487  
 Hills, F. G., 346  
 Hind, H. L., 691, 698  
 Hinsberg, K., 515  
 Hirschfelder, A. D., 476  
 Hirschmüller, H., 284, 333  
 Hobald, K., 590  
 Hobart, F. B., 327  
 Hock, L., 65  
 Hockenyos, G., 93  
 Hoffman, J. I., 346  
 Hofmann, K. A., 590  
 Holcomb, R., 672  
 Holdt, P. C., 94  
 Hollings, P., 634, 644, 645  
 Holmes, W. C., 664  
 Homfray, I., 130  
 Hoover, C. R., 108  
 Hopkins, B. S., 376  
 Hopkins, E. G., 36, 72  
 Hopkins, E. S., 538  
 Horn, D. W., 81, 83, 86, 87  
 Horn, M., 64  
 Horst, F. W., 139  
 Hortala, A., 58  
 Hostetter, J. C., 307  
 Hottes, C. F., 93  
 Hough, W. A., 343, 474, 541  
 Houlton, H. G., 519  
 Howard, P. L., 348  
 Howland, J., 458, 469  
 Hoyt, L. F., 31  
 Hudson, J. H., 594  
 Huelsen, W. A., 93  
 Hughes, E. B., 153  
 Hulbert, R., 543  
 Hurd, L. C., 163, 383  
 Hurley, F. H., 410  
 Hurley, W. B., 39  
 Hurlley, W. H., 551  
 Hussey, R. E., 653  
 Hutchins, L. M., 136  
 Hutchins, W. D., 21  
 Huttig, G. F., 256  
 Hüttner, C., 145, 307, 321  
 Hyuga, A., 16  
 Ibanez, O. G., 687  
 Ilosva, L. I., 644



- Imbert, H., 157  
Imbert, R., 157  
Indovina, R., 546  
Iokhel'son, D. B., 626  
Irish, O. J., 497  
Isaacs, L., 523  
Isaacs, M. L., 140, 534  
Isham, R. M., 359, 360  
Ivanov, V. N., 206, 423  
Ives, F. E., 25
- Jablczynski, K., 83  
Jackerott, K. A., 212, 215, 216, 221  
Jackson, D. D., 611, 653  
Jackson, P. B., 238  
Jacobs, H. R. D., 436  
Jacobs, K. D., 577  
Jakuschoff, P., 95  
Jalusburger, F., 513  
James, L. H., 383, 385, 386  
Jamieson, I. M., 289  
Jandler, G., 433  
Janistyn, H., 25  
Janke, A., 71  
Jannek, J., 605  
Janzig, A. C., 339  
Jarach, M., 392  
Jaumain, D., 711  
Jean, M., 185  
Jelley, E. E., 411  
Jendrassik, L., 506  
Jensen, O. G., 694  
Jlinski, M., 323  
Johns, C. O., 126  
Johnson, A. H., 153, 160, 164, 693  
Johnson, C. M., 102  
Johnson, C. R., 409, 531  
Johnson, G. A., 651  
Johnson, M. O., 126  
Johnston, C. G., 669  
Jolles, A., 480, 517, 518, 522  
Jones, B., 163, 199  
Jones, C. O., 212, 213  
Jones, E. G., 323  
Jones, G. W., 64, 113, 632  
Jones, H. L., 693, 694  
Jones, H. O., 317  
Jones, J. E., 693
- Jones, L. A., 7, 55  
Jones, R. A., 588  
Jones, R. M., 190, 230, 336, 531, 672  
Jones, W. C., 227, 228  
Jordan, L., 182  
Jorgensen, H., 70, 71  
Josland, S. W., 468  
Judd, A. F., 247  
Judd, D. B., 33  
Juncck, 590  
Jungkunz, R., 138  
Jurgens, E., 196
- Kaehn, H., 258  
Kahane, E., 498, 612  
Kahane, M., 498  
Kahler, H. L., 95  
Kahn, B. S., 128, 455, 457, 623  
Kahn, F. L., 590  
Kahn, G., 695  
Kalichevsky, V. A., 58  
Kalina, A., 618  
Kallab, F. V., 22  
Kameda, T., 684, 694  
Kameyama, K., 214  
Kappová, A., 491, 492  
Karns, G. M., 551  
Karpenko, V., 153, 160, 164  
Karshan, M., 506, 513  
Karssen, A., 489  
Kasline, C. T., 66  
Kassler, J., 388  
Kast, H., 115, 116  
Kaufmann, E., 62  
Kaufmann, H. P., 64  
Kay, H. D., 78, 488  
Kayler, H. L., 615  
Kazarinova, V. A., 436  
Kehrmann, F., 638  
Kellin, D., 311  
Kelley, E. G., 62  
Kellogg, J. W., 497  
Kelmperer, R. L. v., 26  
Kemmerer, G., 236  
Kemmerer, K. S., 95, 611  
Kennedy, R. P., 291, 293, 298  
Kenny, W. R., 678  
Kerr, H. W., 688

- Keuffel, K. W., 28  
 Khlopin, V. G., 425  
 Kill, W. E., 300  
 Killan, J. H., 78  
 Kilpatrick, M., 667  
 King, A. A., 238  
 King, C. S., 152  
 King, E. J., 506, 517, 518, 519  
 King, N. J., 688  
 King, W. B., 246  
 King, W. J., 41, 381, 383  
 Kinsley, C., 57  
 Kleimann, H., 409, 453, 513  
 Kleinmann, H., 49, 51, 93, 159, 227, 228, 241  
 Klett, R. E., 47, 55  
 Klinke, J., 159  
 Klockmann, R., 79  
 Klostermann, M., 200  
 Knippenberg, E., 271, 466  
 Knorre, G. von, 323  
 Knowles, H. B., 261  
 Knudson, A., 488, 497, 504  
 Kober, P. A., 91, 92, 93, 241, 409, 511, 513  
 Koch, A. A., 577  
 Kocour, C., 16  
 Koenig, P., 279  
 Koenigsberger, J., 55, 291  
 Kofman, T., 58  
 Kohn-Abrest, C., 178  
     , P. ....  
     on, I. M., 53, 65, 71, 270, ,  
     399, 409, 439, 440, 476, 477, 542,  
     549, 582, 636, 640, 668, 669, 673,  
     682, 684, 685, 694  
 Kolnitz, H. von, 225, 235, 236, 551, 556,  
     557, 567, 569  
 Komar, N. P., 39  
 König, J., 272  
 Kopp, E., 638  
 Kopperl, W., 53  
 Kordatzki, W., 63  
 Korenman, I. M., 333  
 Kraemer, E. O., 202, 203  
 Krajci, L. E., 506, 513  
 Kramer, B., 433, 434, 436, 469, 689  
 Krans, E. W., 199  
 Krantz, Jr., J. C., 96  
 Krasnow, F., 506, 513  
 Krauss, R. B., 564  
 Krepelka, J., 618  
 Kriss, L., 453, 475  
 Kroepelin, H., 65  
 Kropacsy, S., 71  
 Kropf, A., 367, 370  
 Krumholz, P., 310, 377, 480  
 Kühn, I., 689  
 Kupper, 590  
 Kunzmann, T., 15  
 Kurmies, B., 474  
 Kuttner, T., 64, 456, 457, 458, 500, 506  
 Labat, A., 548  
 Lachale, C. E., 226, 598  
 Lachs, H., 290  
 Laget, L., 166  
 Laidlaw, P. P., 465, 478  
 Laing, M. E., 668  
 Lamb, A. B., 108, 409, 532  
 Lamb, F. B., 203  
 La Motte Chemical Products Co., 681  
 Lampe, B., 69  
 Lampe, W., 62  
 Lampitt, L. H., 153  
 Lamplough, F. E., 78  
 Landt, E., 57, 71  
 Lang, K., 515, 609, 624  
 Lange, B., 57  
 Lange, N. A., 567  
 Lanik, J., 491, 492  
 Lapin, L. N., 300  
 Laporte, C. E., 221  
 Larose, E., 452  
 Larsson, E., 717  
 Laszlo, T., 292  
 Latta, J. E., 600  
 Lavollay, J., 312, 473  
 Law, D. J., 278  
 Leavell, G., 298  
 Lebediantzev, A., 497  
 Lebermann, F., 62, 435  
 Le Chatelier, H., 70, 103  
 Lee, F. A., 593  
 Leeper, G. W., 284  
 Legendre, R. A., 17, 65

- Lehmann, P., 288  
Lehner, V., 363, 364  
Leiberson, A., 328  
Leiboff, S. L., 15, 128, 515, 623  
Leitmeier, H., 500  
Leitz, Inc., E., 55  
Lejeune, 358  
Lematte, L., 612  
Lematter, L., 498  
Leonard, C. S., 212  
Leopoldi, G., 168, 202  
Lepierre, C., 480  
Letts, E. A., 638, 648  
Leuchs, F., 258  
Leuchs, H., 258  
Leuchs, W. R., 258  
Levine, M., 209  
Lewis, A. H., 433, 501  
Lewis, E. J., 189  
Lewis, R. C., 12  
Liehenstein, L., 500  
Liebknecht, O., 193, 517, 518, 519  
Likiernik, A., 501  
Lincoln, A. T., 480, 520  
Lindow, C. W., 160, 161  
Lindsay, W. G., 593, 594  
Lindsey, A. J., 226  
Lindt, I. V., 313  
Lisse, M. W., 694  
Livingston, B. E., 136  
Lloyd, L. L., 182  
Lockemann, G., 608  
Logoniv, M. E., 280  
Long, J. H., 75  
Loofmann, H., 267, 474, 501  
Lopez, R. C., 546  
Lorber, L., 300, 618  
Lorenz, R., 548  
Losana, L., 22, 392, 500, 503  
Louise, H. W., 432  
Lovibond, F. E., 34  
Lovibond, J. W., 19, 20, 34, 77, 85,  
Low, Jr., G. W., 409  
Lowe, F., 26  
Lowe, J. T., 110  
Lowry, T. M., 33  
Lubs, H. A., 671, 678, 681  
Lucas, C. C., 202, 203, 519  
Lühr, W., 225  
Lukiesh, M., 81  
Lum, E. A., 328  
Lundell, G. E. F., 261, 346, 497  
Lundgren, H. P., 62  
Lunge, G., 338  
Lutz, R. E., 352  
Lyman, C. M., 671  
Lyman, H., 449, 450  
Lynch, G. R., 203  
Lyons, E., 263, 298  
  
Maag, O. L., 333, 387  
Machefoeuf, A., 188  
Machelboeuf, M., 497  
Mackay, J. G., 613  
Maechling, E. H., 231, 244  
Magnin, G., 126  
Magnus, H., 55  
Mahr, C., 212, 220, 221  
Mai, J., 248  
Maingard, J., 179  
Mainzer, F., 695  
Majer, V., 179, 181  
Makishima, S., 214, 254  
Makris, K. C., 659  
Malatesta, G., 404  
Malengreau, F., 222  
Malmros, H., 305  
Malossi, L., 223  
Malowan, S. L., 333  
Malyarov, K. L., 439, 551  
Manley, C. H., 226  
Manley, J. J., 62  
Manin, W., 33, 275, 276  
Manneback, O., 64  
Marcal, J. M. deC., 678  
Marchling, E. H., 216  
Marenzi, A. D., 437  
Markova, G. A., 500, 507  
Marmoy, F. B., 433, 501  
Marriott, W. M., 117, 283, 458, 469  
Marsh, F. W., 94  
Marshall, H., 332, 339  
Marshall, J., 622  
Marshall, J. T. W., 91  
Martin, F., 247  
Martland, M., 497

# AUTHOR INDEX

- Martz, R. J., 693  
 Marx, A., 689  
 Mathison, G. C., 487  
 Matskievich, V. B., 551  
 Maxfield, L. S., 258  
 Maxson, R. N., 402, 406  
 Mayer, K., 63  
 Mayer, O., 288, 289  
 Mayrand, L. P., 227  
 Mazzucchelli, A., 424, 426  
 McBain, J. W., 668  
 McCabe, C. R., 358, 362, 367, 368, 369  
 McCance, R. A., 439, 440  
 McCandless, J. M., 382  
 McCandlish, D., 65  
 McCarthy, W. J., 199  
 McClean, A. P. D., 104, 120  
 McClendon, J. F., 551, 552, 553, 554, 556,  
     557, 567, 569, 711  
 McCollam, C. H., 383, 387  
 McCollum, E. V., 577  
 McCracken, R. F., 36, 72, 338  
 McCrae, J., 53, 704  
 McCrumb, F. R., 678  
 McFarlane, W. D., 164, 166  
 McHargue, J. S., 554, 560, 574  
 McHatton, L. P., 21, 33  
 McIlvaine, T. C., 674  
 McInerney, T. J., 690  
 McKibbin, R. R., 672  
 Mclean, S. P. D., 65  
 McMamee, P. C., 543  
 McMaster, L., 447  
 McNicholas, H. J., 72  
 McQuarrie, I., 696  
 Meade, G. P., 15  
 Mears, B., 653  
 Meaurio, V. L., 372  
 Mecklenburg, W., 593  
 Medigreceanu, F., 337  
 Meerburg, P. A., 551, 552  
 Mees, C. E. K., 8  
 Mehlig, J. P., 69, 71  
 Mehurin, R. M., 147, 148, 168, 230  
 Meimberg, E., 401  
 Meinck, F., 64  
 Meissner, O., 96  
 Meldrum, R., 348  
 Meldrum, W. B., 409, 532  
 Mellon, M. G., 66, 69, 71, 519, 520, 670  
 Mellor, J. W., 285, 297, 328, 365  
 Mérière, P., 180  
 Merejkovsky, B. K., 404, 406  
 Merwin, H. E., 356, 571  
 Messinger, L., 439, 442  
 Metzler, A., 590  
 Meulengracht, E., 62  
 Meyer, A. H., 244, 500, 508, 509, 510,  
     523  
 Meyer, J., 604, 605  
 Micewitz, S., 638  
 Michaelis, L., 298, 329, 536  
 Michel, A., 294, 304  
 Mickwitz, A., 320, 330  
 Middleton, A. R., 261, 399  
 Mikhalechishin, G. T., 532  
 Miller, C. W., 497, 500  
 Miller, E. H., 647  
 Miller, E. J., 203  
 Miller, R. C., 669  
 Millikan, G. A., 58  
 Milton, R., 611  
 Mines, G. R., 12, 47  
 Mingaye, J. C. H., 419  
 Minglais, H., 497  
 Minot, A. S., 337, 339  
 Mirescu, J., 569  
 Mission, G., 597  
 Misson, G., 485  
 Mitchell, C. A. D., 261  
 Modzelewski, T., 117  
 Moffatt, M. R., 205  
 Mohler, H., 345, 620  
 Moilliet, J. L., 409  
 Moir, J., 403  
 Monnier, A., 590  
 Monthignie, E., 389  
 Moody, M., 26  
 Moore, M. C., 165  
 Moreau, E., 64, 65  
 Moreau, L., 247  
 Morgan, M. F., 637, 717  
 Morgan, W. V., 206  
 Morgulis, S., 624, 625  
 Morris, V. H., 430  
 Morth, H., 210, 304, 333

- Moseley, H. W., 165  
Moser, L., 399  
Motherwell, H. A. B., 213  
Moulin, A., 198, 279  
Mousseron, M., 437, 461, 462, 463  
Muer, H. F., 538, 540, 611, 613  
Mulder, P. J., 57  
Müller, 394  
Muller, A., 406  
Muller, H., 200  
Müller, H. T., 65  
Muller, J. H., 364  
Muller, L., 491  
Müller, R. H., 57, 58  
Mullershossly, E., 55  
Mummery, W. R., 305  
Munro, L. A., 500  
Munsell, A. A., 72  
Munsell Color Co., 72  
Munsell, J., 502  
Muntwyler, E., 711  
Murray, C. W., 230  
Murray, J. K., 690  
Musakin, A. P., 259  
Myasnikova, A. M., 438  
Myers, C. N., 199  
Myers, V. C., 53, 55, 704, 711  
Mylus, F., 424, 426, 438
- Naeser, G., 22  
Naumann, E., 266  
Neahr, W. C., 63  
Neave, S. L., 646  
Necke, A., 200  
Nelson, G. H., 209  
Némec, A., 430, 491, 492  
Nenadkevich, K. A., 333  
Neuber, F., 180  
Neurath, F., 480, 517, 522  
Newcomb, C., 335  
Newcomer, H. S., 22  
Newell, I. L., 258  
Newman, A., 497  
Newman, R. K., 206  
Nicholls, J. R., 190  
Nichols, C. M., 539  
Nichols, M. S., 633  
Nickolls, L. C., 213
- Niessner, M., 399  
Ninegar, C. H., 16  
Nitzescu, I. I., 161  
Noel, 358  
Noponen, G. E., 640  
Nordlander, B. W., 184, 185  
Norton, J. F., 644, 645  
Nowak, C. A., 29  
Nowicki, R., 111  
Noyes, A. A., 208, 233  
Noyes, H. A., 630  
Noyes, H. M., 70, 81  
Noyes, W. A., 355
- Oberhauser, F., 523, 525  
Obermer, E., 611  
Ochotin, V. P., 315  
Odling, W., 66  
Offerman, 577  
Offord, H. R., 589  
Okhotin, V. P., 260  
Olszewski, 343  
Oosting, W. A. J., 689  
Oppenheimer, E., 545  
Oreutt, F. S., 117  
Orelkin, B., 308  
Oshima, F., 160  
Osler, T. G., 203  
Osmund, F., 500  
Ossin, H., 283  
Osterberg, A. E., 241  
Osterburg, F., 258  
Ostwald, W., 72, 96
- Page, H. J., 572  
Palmer, A. E., 646  
Palmer, A. W., 650  
Palmer, R. M., 226  
Pamfil, G. P., 356  
Panchenko, G. E., 384  
Pangritz, F., 227, 228, 241  
Park, B., 189, 214, 253, 256, 384  
Parker, A. J., 689  
Parker, F. W., 500  
Parker, W. L., 64, 632  
Parks, A. M., 12  
Parks, H. C., 94  
Parr, S. W., 95

- Parri, W., 523, 524  
 Parsons, L. B., 669  
 Parsons, L. W., 31  
 Partridge, H. M., 57  
 Passamaneck, E., 338  
 Passerini, N., 500  
 Paterson, W., 63  
 Patterson, W. L., 22, 49, 65  
 Pavelka, F., 210, 304, 333, 453, 475  
 Paviot, J., 302  
 Pavlimova, A. V., 548  
 Payne, W. W., 465, 478  
 Peack, E. S., 246  
 Pearson, R. W., 164  
 Peebles, A. R., 12  
 Peet, G. D., 63  
 Pellin, F., 65  
 Pena, A., 490  
 Pensa, A. J., 128  
 Perry, I., 27  
 Perry, J., 57  
 Perry, J. W., 65  
 Pestov, N. E., 75, 85  
 Peterfi, T., 696  
 Peterman, F. I., 259  
 Peters, B. G., 63  
 Peterson, W. H., 117, 333  
 Petit, P., 691  
 Petrie, A. H. K., 501  
 Petrov, A. D., 201  
 Petrovskii, A., 376  
 Pett, L. B., 502  
 Peyrot, E., 571  
 Pfeiffer, G., 511, 560  
 Pfeilsticker, K., 639  
 Phelps, E. B., 151, 650  
 Phelps, R. H., 539  
 Phillips, H., 151, 152, 294  
 Pickard, J. A., 367, 371  
 Pickard, P., 332  
 Pickardt, E. G., 66  
 Pien, J., 247  
 Pierce, L. F., 695  
 Pierre, T., 176  
 Pierre, W. H., 682, 689  
 Pierson, G. G., 250, 407, 420, 568, 607  
 Pilgrain, P., 157  
 Pincussen, L., 187, 346, 513  
 Pinkus, A., 332  
 Pinsl, H., 517  
 Pirlot, J. M., 330, 435  
 Poch, P., 589  
 Poirot, G., 221  
 Polinkavskii, A. I., 464  
 Pollard, W. B., 408  
 Popp, H., 231, 232  
 Porter, L. E., 539  
 Potapov, A. I., 262  
 Potschinok, Ch. N., 502  
 Pouget, I., 511  
 Pouzergues, J., 221  
 Powell, A. D., 327, 328  
 Powers, E. B., 63, 117, 119  
 Pozzi-Escot, M., 406  
 Pratt, D. S., 629, 633, 634  
 Prideaux, E. B. R., 663, 669, 685  
 Priest, I. G., 7, 32, 33, 72  
 Prinsen-Geerligs, H. C., 15, 65  
 Prister, A., 403  
 Proctor, H. R., 34  
 Prodam, L., 176  
 Pugh, A. J., 491  
 Pulfrich, C., 96  
 Pulsifer, H. S., 283, 303, 304  
 Pyriki, C., 186  
 Quimby, E. H., 569  
 Race, C. N., 57  
 Radiovisor Parent, Ltd., 57  
 Ram, K., 156, 157  
 Ramage, W. D., 669  
 Ramakers, L., 332  
 Ramann, E., 678  
 Ramsay, W., 130  
 Randall, M., 186  
 Randles, F. S., 488, 497, 504  
 Rao, B. S., 141  
 Raper, A. S., 497  
 Rasmussen, H. B., 212, 215, 216, 221  
 Razek, J., 57  
 Rea, F. W., 638, 648  
 Redfield, H. W., 629, 633, 634  
 Reed, L., 618  
 Rees, R. L., 21  
 Rehling, C. J., 689

- Reimann, C. K., 337, 339  
Reimann, F., 594  
Reimann, S. P., 57  
Reis, F., 305  
Reith, J. F., 194, 225, 557  
Remington, R. E., 153, 154, 160, 164, 225,  
235, 236, 551, 553, 554, 556, 557,  
567, 569  
Renss, A., 288  
Revol, L., 302  
Revzyuk, B. A., 65  
Rey, L., 345  
Reynold, D. S., 577  
Reynolds, F., 383  
Ribereau-Gayon, J., 294  
Richards, M. B., 333  
Richards, O. W., 717  
Richards, T. W., 91, 409, 531  
Richardson, F. W., 33, 149, 275, 276, 634,  
644, 645  
Richardson, G. M., 298  
Richardson, J. R., 348  
Rice, C. S., 695  
Richmond, H. D., 226, 654  
Richter-Quittner, M., 497  
Rideal, S., 133  
Ridgeway, R., 72  
Riegler, E., 502  
Riehm, H., 492, 638  
Riffart, H., 62  
Rimington, C., 504  
Ring, C. H., 66, 68, 79, 256, 657  
Rippart, J., 65  
Risch, C., 71  
Roberts, H. F., 22  
Roberts, N., 43, 53, 75, 85  
Robertshaw, G. F., 688  
Robinson, F. C., 427  
Robinson, H. M., 203  
Robinson, H. W., 669  
Robinson, R. J., 509, 651, 654  
Robinson, W. O., 233, 234, 235, 501, 606  
Robison, R., 488, 497  
Roe, J. H., 455, 457, 497  
Roepke, R. R., 496  
Rogers, H. W., 261  
Rohwer, A. G., 165  
Roller, P. S., 262  
Rollet, A. P., 314  
Romijn, G., 645  
Rona, P., 453  
Roos, O., 689  
Rose, A. R., 99  
Rosenblatt, M., 337  
Rosenblatt, T., 527  
Rosenheim, O., 20  
Rosenkränzer, F., 593  
Rosin, J., 73  
Ross, E. M., 95, 615  
Ross, J. R., 202, 203  
Ross, W. H., 190, 230, 336, 531, 571, 577,  
672  
Rost, C. O., 332, 335, 341  
Roth, R., 590  
Rowell, H. W., 212  
Roy, W. R., 560  
Rozanov, S. N., 334, 436  
Rufe, R., 557  
Rupp, R. E., 149  
Russell, S., 711  
Ruthing, A., 144  
  
Sacher, J. F., 284  
Sagrista, J. N., 602  
Sakurada, H., 532, 563  
Salisbury, H. M., 682  
Salit, P. W., 440  
Sallinger, H., 678  
Salter, R. M., 630  
Sammatt, C. F., 22, 62  
Sammis, J. L., 690  
Sanchis, J. M., 581  
Sandra, K., 57  
Sanders, G. P., 450, 476  
Sanger, C. R., 236  
Sanger-Schepherd, E., 56  
Santschi, F., 690  
Sarata, U., 166  
Sarquis, M. N., 186  
Sasaki, R., 627  
Satterlee, H. S., 230  
Sauer, H., 95  
Savchenko, D. S., 259  
Sayers, R. R., 113, 114  
Sazavsky, V., 53  
Sazerac, R., 221

- Scales, F. M., 94, 636  
 Searritt, E. W., 502, 508  
 Seazzola, R., 241, 512  
 Seazzola, U., 241, 512  
 Schaeffer, J. A., 286  
 Scharrer, D., 311  
 Schell, E., 21  
 Scherer, Jr., P. C., 598  
 Scheringa, K., 289  
 Schlegel, J. W., 667  
 Schlesinger, E., 48  
 Schlesinger, F., 437  
 Schlesinger, H. I., 83  
 Schlesinger, H. L., 284  
 Schluty, M., 247  
 Schmidt, A., 115, 116  
 Schmidt, F., 93  
 Schmidt, P., 200  
 Schmegg, H., 55  
 Schneider, H., 65  
 Schoenberg, V. A., 64  
 Scholtz, A., 62  
 Scholz, W., 284  
 Schönheimer, R., 160  
 Schoonover, J. W., 712, 713, 715  
 Schoorl, N., 150, 151, 196  
 Schormüller, J., 523, 525  
 Schott, F., 146  
 Schou, S. A., 212, 215, 216, 221  
 Schreiner, O., 42, 463, 470, 480, 483, 520,  
     611, 634  
 Schrenk, H. H., 236  
 Schricker, J. A., 225, 245, 501  
 Schröder, H., 225  
 Schroder, R., 485  
 Schroeder, A., 34  
 Schubert, M. P., 436  
 Schultz, E., 490  
 Schultz, E. W., 689  
 Schultz, G., 280  
 Schultze, K., 116  
 Schuster, E. H. J., 20  
 Schuyten, M. C., 648  
 Schwartz, M. C., 518  
 Schwartz, R., 355  
 Schwartz, E. W., 262  
 Seohy, 358  
 Scott, A. F., 409, 410  
 Scott, L. H., 95  
 Scott, W. M., 57  
 Scott, W. W., 147, 152, 186, 192, 218, 527,  
     528  
 Scotti, G., 523, 524  
 Seaborne, F. S., 418, 419, 420  
 Seifert, R., 62  
 Seiser, A., 200  
 Selle, H., 115  
 Sendroy, Jr., J., 712  
 Serles, E. R., 476  
 Shakhkeldian, A. B., 162  
 Sharp, C. H., 57  
 Sharp, P. F., 690  
 Sharpe, J. S., 463  
 Sharpe, W. F., 36, 72  
 Sharpless, G. R., 577  
 Shaw, P. A., 207, 209  
 Sheaff, H. M., 138  
 Sheaff, P. A., 62  
 Sheen, R. T., 95, 615  
 Sheets, O., 164  
 Sheftel, A. G., 47  
 Shemyakin, F. M., 366  
 Sheppard, S. E., 94, 594  
 Shipp, H. L., 439, 440  
 Shmuk, H., 632  
 Shneerson, S. B., 496  
 Shohl, A. T., 696  
 Shpak, M. A., 266  
 Shub, M. E., 253, 254  
 Shubaev, 266  
 Shuey, G. A., 577  
 Shulenberger, F. W., 64  
 Siemssen, J. A., 405  
 Simonot, E., 156  
 Simpson, T. D., 21  
 Singleton, W., 376, 380, 381, 383, 423  
 Sinyakova, S. I., 185  
 Sisco, F. T., 143  
 Sisley, W. P., 175  
 Sittrich, M., 275  
 Sjostrom, O. A., 688  
 Skinner, J. T., 333  
 Skopp, E., 472  
 Skrimshire, G. H., 168, 170, 187, 188  
 Slater, R. H., 203  
 Slawik, P., 370



- Smith, E., 694  
Smith, 2nd, F. L., 202  
Smith, G. F., 63  
Smith, H. L., 89, 285, 287, 296  
Smith, H. V., 574  
Smith, L., 638  
Smith, N. K., 698  
Smith, O. M., 440, 583  
Smith, W. C., 155, 295, 338  
Smitt, N. K., 574  
Smoot, C. C., 63  
Snethlage, H. C. S., 635  
Snider, H. J., 498  
Sobel, A. E., 433, 434, 436  
Sola, T., 452, 478  
Solowiejczyk, S., 151  
Sommer, H. H., 160  
Sondén, K., 21  
Sorenson, S. P. L., 84  
Sottery, C. T., 260  
Spackman, L. S., 689  
Spacu, G., 159, 609  
Spear, E. B., 208  
Spencer, G. C., 73, 280  
Spengler, O., 57  
Spiro, H. S., 205  
Sprengels, H., 629  
Spurge, G., 389  
Stadie, W. C., 669  
Stadler, J., 494  
Staiger, 569  
Staley, W. D., 95  
Stanfield, K. E., 384, 385, 386  
Stanford, R. V., 36, 505  
Stankiewicz, W., 83  
Stansby, M. E., 582  
Steenbergen, H. D., 633, 643  
Steenkamp, J. L., 266, 301, 434, 442, 476  
Stefanovski, V. F., 58  
Steffen, F., 437  
Steffens, W., 520  
Steiger, G., 42, 571, 572  
Steiner, O., 390  
Steinhauser, K., 494  
Stenger, V. A., 549  
Sterges, A. J., 629, 633  
Stern, H. T., 678  
Steudel, H., 263  
Stewart, R., 629  
Stich, C., 207, 516  
Stieglitz, E. J., 646  
Stirlen, E. D., 17  
St. Lorant, I., 594  
Stock, A., 178, 181  
Stokes, H. N., 284, 295, 297  
Stokes, Jr., J., 695  
Stone, F. B., 212  
Stoppel, A. E., 385  
Story, B. W., 58  
Strafford, N., 603  
Stratton, R. C., 343, 541  
Stromberg, H., 638  
Stubbs, J. R., 226  
Stueber, A. H., 667  
Stugart, R., 290, 293  
Subbarow, Y., 488, 497, 504, 505, 506  
Suichov, A. P., 315  
Sulkowitch, H. W., 96  
Sullivan, Jr., F. W., 42  
Sullivan, V. R., 63  
Sultzbarger, J. A., 212, 216, 217  
Supplee, G. C., 152  
Sutherland, G., 94  
Swank, H. W., 519, 520  
Sweeney, O. R., 547  
Sweet, W. W., 598  
Szczepanik, J., 48  
Szegoe, L., 283, 577  
Taber, W. C., 227  
Tanahaev, I., 162  
Tanahaev, N. A., 162  
Tananaev, N. A., 384  
Tannahill, R. W., 190  
Tanonief, N. S., 502  
Tapernoux, A., 717  
Tarugi, N., 333, 341  
Tasker, H. S., 317  
Taub, A., 66, 71  
Taubmann, G., 247  
Taylor, A. E., 77, 497, 500  
Taylor, F. H., 433  
Taylor, H. B., 690  
Taylor, H. J., 581, 582  
Taylor, W. A., 34, 546  
Teitelbaum, M., 219, 270, 352, 525, 604

- Thatcher, R. W., 166  
 Thayer, L. A., 84, 517, 518  
 Theis, R. C., 505  
 Theriault, E. J., 538  
 Thiel, A., 26, 62, 65, 71, 669, 677  
 Thiel, W., 62  
 Thilenius, R., 178, 180, 181  
 Thomas, A. W., 88  
 Thomas, B. D., 237  
 Thomas, P., 658  
 Thompson, A., 296  
 Thompson, F., 477  
 Thompson, F. C., 716  
 Thompson, H., 332  
 Thompson, J. G., 94  
 Thompson, P. K., 351  
 Thompson, T. G., 519, 581, 582  
 Thone, B., 199  
 Thornton, Jr., M. K., 600  
 Thornton, W. M., 355, 362  
 Thorogood, A. L., 581  
 Thresh, 519  
 Thresh, J. C., 186, 196  
 Thrun, W. E., 259, 261, 262, 264, 265, 477  
 Tidy, C. M., 66  
 Tintometer, Ltd., 20  
 Tischer, J., 436, 468, 493  
 Tischler, N., 603  
 Tisdale, F. S., 513  
 Tisdall, F. F., 433, 469  
 Tissier, M., 439  
 Title, C., 183  
 Tittsler, R. P., 694  
 Tod, H., 547  
 Toepfer, E. W., 611, 612  
 Toggenburg, F., 39  
 Tolman, R. C., 62, 94  
 Tompsett, S. L., 298  
 Tompson, T. G., 289  
 Tomula, E. S., 328, 437  
 Toussaint, R., 86  
 Tracy, E., 711  
 Tracy, G., 622  
 Travers, A., 375  
 Treadwell, F. P., 570, 577, 654  
 Treadwell, W. D., 494, 501  
 Trebler, H. A., 153, 160, 164  
 Trillat, M. A., 200, 342  
 Trillich, H., 96  
 Troland, L. T., 6, 57  
 Truog, E., 244, 500, 509, 510, 523  
 Tschergichow, J., 395  
 Tschopp, E., 444, 501, 533  
 Tschugaeff, L., 308  
 Tsen, Z. Y., 481  
 Turner, R. G., 551, 566, 567, 569  
 Tuthill, E., 440, 441  
 Twyman, F., 57  
 Uga, Y., 435  
 Ulrich, W., 15, 608  
 Unangst, R. B., 262  
 Underhill, F. P., 259  
 Urbach, C., 454, 468, 486, 508, 510, 608  
 Urbanek, L., 502  
 Urech, P., 491  
 Usher, F. L., 141  
 U. S. Steel Corporation, 102, 103, 334,  
     358, 359, 367, 383  
 Utescher, K., 689  
 Uzel, R., 647  
 Valyashko, N. A., 216  
 Van Alstine, E., 65  
 Van Arnum, W. I., 96  
 van der Luigen, G., 501  
 Van der Vlugt, L. S., 196  
 van de Vlugt, L. A., 288  
 Vangasbecq, L., 294  
 Van Slyke, D., 487  
 Vanslyke, L. L., 689  
 van Oyen, C. F., 693  
 van Urk, H. W., 283, 284, 287, 309, 686  
 Van Valkenburgh, H. B., 83, 284  
 van Wering, C. R., 489  
 Vasarhelyi, B., 501  
 Vasil'ev, A. A., 253, 254  
 Vasil'eva, E. V., 479  
 Veitch, F. P., 480, 520  
 Vellenge, S. J., 539  
 Verbeck, P., 64  
 Viehoever, A., 126  
 Vinet, E., 247  
 Vinograd, M., 228  
 Vinogradov, A. P., 373  
 Virgili, J. F., 588

- Virup, P. K., 216  
Vleeschhouwer, J. J., 673  
Vogelenzang, E. H., 182  
Voigt, K., 96  
Voskresenskaya, D. V., 334  
Vostrebai, J., 384  
  
Wael, H., 516  
Waggoner, C. S., 153  
Wagner, C. R., 571, 577  
Walker, J. K., 33  
Walker, J. W., 130, 132  
Walker, W. B., 285, 297, 304  
Wallace, C. F., 30  
Wallace, G. I., 646  
Walpole, G. S., 84  
Walters, H. E., 334  
Walton, Jr., C. F., 22  
Walton, Jr., J. H., 358, 361, 363  
Walton, S. G., 206  
Wandenbulcke, F., 517, 519  
Wanderscheck, H., 57  
Wandrowski, B., 271  
Ward, A. T., 685  
Ward, L. A., 567  
Ward, T. J., 226, 232, 236  
Ward, R. R., 492, 501  
Warren, R. G., 491, 572  
Watson, F. J., 698  
Watson, F. S., 569  
Wattenberg, H., 651  
Webb, S. K., 527, 528  
Weeks, M. Z., 551, 567  
Weibke, F., 392  
Weil, A., 58  
Weiland, H., 267, 339, 436, 439, 466, 474,  
501, 655  
Weiland, W., 271, 300  
Wein, S., 57  
Weir, J. W., 15  
Weir, P. L., 96  
Welker, W. H., 622  
Weller, A., 355  
Wells, P. V., 409  
Wells, R. C., 91, 356, 409, 531  
Wendehorst, E., 382  
Wenker, H., 678  
Wesson, D., 63, 79  
  
Wester, D. H., 333, 339  
Westfall, B. B., 536  
West-Knights, J., 480  
Weston, R. S., 336, 649  
Weston, V., 690  
Westrip, G. M., 688  
Whalen, F. C., 57  
Wheatley, A. H., 505  
Whelan, M., 638  
Wherry, E. T., 688  
Whipple, M. C., 69  
White, B. S., 148, 286, 287, 297  
White, C. H., 53  
White, E. C., 62  
White, J., 226  
Whitehorn, J. C., 488, 497, 505, 534  
Wichmann, H. J., 202, 571  
Wickers, E., 73  
Widner, R., 345  
Wiegand, J. A., 196  
Wiesler, K., 64  
Wilcox, L. V., 57, 577  
Wilhoit, A. D., 130, 132  
Wilkins, Jr., E. S., 202, 203  
Willard, H. H., 332, 339, 498, 577  
Williams, K. T., 233, 234, 235, 501, 606  
Williams, R. J., 671  
Williams, R. S., 274, 653  
Williams, W., 290  
Willoughby, C. E., 202, 203  
Wilson, E. G., 244, 517  
Wilson, P. W., 117  
Wilson, R. E., 31  
Wilson, R. P., 21  
Winkler, B., 258  
Winkler, L. W., 136, 138, 195, 288, 306,  
346, 520, 596, 635, 654  
Winkler, M., 5  
Winogradowa, E. N., 569  
Winter, I. B., 295  
Winter, O. B., 203, 261, 262, 264, 498, 577  
Winzer, R., 178, 180, 181  
Wirsing, F. H., 317  
Wirth, H. E., 509, 651, 654  
Withrow, J. R., 547  
Wöhler, L., 418, 419  
Wolesensky, E., 612

- Wolf, C. G. L., 283  
Wolffenstein, R., 499  
Wolker, W., 472  
Wollmer, W., 55  
Wolman, A., 687  
Wong, S. Y., 291  
Wood, J. T., 278  
Woodard, H. Q., 569  
Woodman, A. G., 365, 373, 480, 519, 520,  
644, 645  
Woodman, A. S., 21  
Woodward, G. E., 713, 715  
Wrigge, F. W., 392  
Wright, W. H., 65  
Wu, H., 48, 183, 461, 500, 534, 654  
Wulff, P., 717  
Wuth, O., 546  
  
Yamaguchi, S., 329  
Yamamoto, T., 223  
Yamazaki, J., 623  
Yant, W. P., 110, 113, 114  
  
Yoe, J. H., 15, 42, 58, 259, 265, 295, 296,  
302, 317, 369, 536, 590  
Yoshimatsu, S., 435, 443, 459, 471, 533,  
535, 563, 621  
Young, D. W., 554, 560  
Youngburg, G. E., 245, 508  
Yudnich, T., 439  
Yutzy, H., 409  
  
Zahnd, H., 612  
Zeiss, Inc., C., 47  
Zerban, F. W., 26  
Zert, K., 32  
Zimmermann, W., 178, 181  
Zinzadze, C., 57, 244, 433, 434, 437, 500,  
501, 503, 506  
Zinzadze, S. R., 244  
Zondek, H., 548  
Zubareva, N., 260  
Zucker, T. F., 490  
Zverev, V. S., 517  
Zvyagintzev, O. E., 428  
Zwilling, G., 497

# SUBJECT INDEX

- Absorbent** solution for carbon dioxide, 120, 122
- Acetone-water mixtures, hydrogen-ion concentration in, 688
- Acetylacetone, iron by, 303
- Acid, battery, iron in, 287
- Acid ashing of organic samples, 497
- Accuracy, general, 74
- limitations of, 74
- series of standards method, 74
- dilution method, 74
- balancing method, 74
- duplication method, 74
- sources of error, 74
- Acid sodium thiosulfate, arsenic by, 249
- Active oxygen, by citric acid and ammonium molybdate, 138
- Adurol, oxygen by, 136
- Agar, nutrient, hydrogen-ion concentration in, 694
- Air, carbon monoxide in, 112, 116
- chlorine in, 539
- mercury in, 179
- Alizarin, calcium by, 465
- Alizarin-S, aluminum by, 259
- Alkali, standard, bottle for, 673
- Alloxantin, iron by, 310
- Alloys, aluminum in, 262
- antimony in, 253
- bismuth in, 218
- iron in, 301, 307
- nickel in, 315
- tungsten in, 377
- Aluminon. See aurin tricarboxylic acid
- Aluminum, apparatus for development of aurin tricarboxylic acid lake, 264
- artificial standard, calibration curve for, 264
- as oxide by alizarin-S, 259
- by alizarin-S, 259
- by aurin tricarboxylic acid, 260, 265
- by cupferron, 269
- by eriochrome cyanine, 271
- by hematoxylin, 266
- by hydroxymethylanthraquinone, 271
- by 8-hydroxyquinoline by conversion to a dye, 267
- by phosphomolybdotungstic acid, 270
- by quinalizarine, 270
- in alloys, 262
- in brass, 262
- in bronze, 262
- in lead-base bearing metals, 262
- in phosphor-bronze, 262
- in spelter, 262
- in tin-base bearing metals, 262
- in tissue, 262, 263
- in water, 265
- phosphorus in, 494
- separation from iron, 266
- standards for, 260, 265, 266, 270
- Aluminum oxide, in aluminum, 260
- Amaranth, arsenic in, 232
- p*-Aminodimethylaniline, sulfides by, 593
- 1,2,4-Aminonaphthol sulfonic acid, calcium by, as the phosphate, 455
- Ammonia, by Nessler's reagent, 645
- by phenol and sodium hypochlorite, 658
- by silver nitrate and tannin, 659
- hydrocyanic acid as, 127
- in blood, 652
- in feed, 653
- in milk, 652
- in sea water, 651
- in sewage, 651, 652
- in steel, 653
- in urine, 652
- in water, 649, 650
- nephelometrically by a modified Nessler's reagent, 657
- standards for, 655, 658
- Ammonia-free water, 649

- Ammoniacal silver nitrate solution, as reagent for carbon monoxide, 115, 116
- Ammonium ferrocyanide, magnesium by, 475
- Ammonium molybdate, hydrogen peroxide by, 140
- tin by, 256
- titanium by, 365
- vanadium by, 372
- Ammonium molybdate and citric acid, oxidizing power by, 138
- Ammonium thiocyanate. See thiocyanate
- Amyl alcohol-ether, extraction of iron by, 297
- Aniline hydrochloride, 588
- chlorates by, 588
- Animal matter, fluorides in, 585
- Antimony, as sulfide, 251
- by phosphomolybdotungstic acid, 254
- by pyridine and iodide, 253
- extraction with amyl alcohol, 254
- in alloys, 253
- in brass, 251
- in copper, 251
- in organic matter, 252
- removal on copper foil, 251
- separation from other metals, 251
- standards for, 252
- Antimony ore, bismuth in, 213
- Apparatus, for balancing methods, 38
- for comparison with series of liquid standards, 10
- for comparison with solid standards, 19
- for dilution methods, 35
- for duplication methods, 36
- Applications of nephelometry, 93
- Aqueous humor, copper in, 161
- Arsenic, as arsine. See Gutzeit method.
- as trisulfide, 248
- apparatus for electrolytic Gutzeit method, 239, 240
- apparatus for Gutzeit method, 236, 237
- by acid sodium thiosulfate, 249
- by a modified electrolytic Gutzeit method, 240, 241
- by cocaine-molybdate reagent, 241
- by electrolytic Gutzeit method, 239, 240
- by formation of molybdenum blue, 244
- by Gutzeit method, 225
- by mercurous chloride, 250
- by quinine arsenomolybdate, 242
- by silver nitrate, 247
- by sodium hypophosphite, 243
- by stannous chloride, 246
- by strychnine-molybdate reagent, 241
- distillation of, 233
- apparatus for, 233
- in amaranth, 232
- in biological material, 230
- in blood, 231
- in blood serum, 228
- in bones, 232
- in coal, 238
- in combustible materials, 235
- in copper, 230
- in dry substances, 227
- in dyes, 232
- in erythrosine, 233
- in food dyes, 232
- in fruit peelings, 230
- in gelatine, 230
- in grain, 232
- in guinea green B, 232
- in hops, 232
- in inorganic samples, 227
- in light green S F yellowish, 232
- in liquids, 227
- in malt, 232
- in naphthol, 232
- in oils, 236
- in organic samples, 228, 235, 239
- bomb decomposition of, 229
- Carius oxidation of, 228
- dry ashing of, 235
- in phosphoric acid, 230
- in pulped vegetables, 227
- in pyrites, 234
- in soils, 233
- in sprayed foliage, 232
- in sugar, 232
- in sulfides, 234
- in tartrazine, 232
- in tissue, 230, 235, 239

- Arsenic, in tobacco, 231  
in vegetable matter, 235  
in water, 235  
in wine, 247  
in yellow S, 232  
iron content of sample high, 245  
mercuric bromide paper for, 236  
phosphorus absent, 245  
phosphorus present, 245  
samples ignited with magnesium nitrate, 245  
standards for, 238, 246, 248, 249
- Arsenic ore, bismuth in, 213
- Arsenic pentoxide, as standard for arsenic, 246
- Arsenious oxide, as standard for arsenic, 238, 248, 249
- Arsenious sulfide, sulfides as, 597
- Arsenophosphotungstic acid, 329  
cobalt by, 328
- Artificial liquid standards, 66. See also standards.
- Ash, sodium in, 441
- Ashing organic samples, by acid ashing, 497  
by bomb decomposition, 229  
by Carius oxidation, 228  
by dry ashing, 235, 496  
by perchloric acid, 498  
by peroxide, 499  
by wet ashing, 497
- Aurin tricarboxylic acid, aluminum by, 260, 265  
beryllium by, 399
- Bader colorimeter, 30
- Baking powder, lead in, 186
- Balancing method, 1, 38  
accuracy of, 74  
calculation for, 98
- Barium, as the sulfate, 467
- Battery acid. See acid.
- Beer's law, 3  
application of, 4  
mathematical development, 3  
modification of, 5
- m*-Benzaminosemicarbazide, copper by, 166
- Benzidine, chlorine by, 541  
copper by, 162  
iridium by, 425  
manganese by, 343  
phosphorus by, as phosphomolybdate, 507
- Beryllium, by aurin tricarboxylic acid, 399  
by curcumin, 398  
by quinalizarine, 399  
separation from aluminum, 399  
standard for, 398
- Bichromatic solution, apparatus for measuring color of, 47, 49  
error from comparing, 88
- Bile, phosphorus in, 488  
thiocyanate in, 610
- Biological materials, arsenic in, 230  
copper in, 160, 161  
hydrogen-ion concentration in, 695  
iron in, 312  
micro method for, 312  
nickel in, 315  
sulfates in, 612
- Bismuth, as iodide, 212  
as iodobismuthate of quinine, 221  
as sulfide, 222  
by cinchonine potassium iodide, 218  
by *o*-hydroxyquinoline, 221  
by phosphomolybdotungstic acid, 219  
by potassium thiocyanate, 222  
by sodium stannite, 223  
by thiourea, 220  
in alloys, 218  
in antimony ore, 213  
in arsenic ore, 213  
in blood, 216  
in bone, 216  
in copper, 213, 224  
in copper ore, 213  
in copper solutions, 214  
in feces, 216  
in inorganic samples, 213  
in lead, 214  
in lead alloys, 214  
in lead ore, 213  
in ores, 218  
in organic samples, 215, 223

- Bismuth, in silver ore, 213  
in tin ore, 213  
in tissue, 216  
in tissue and organs without ashing, 216  
in urine, 215, 217  
iron in, 287  
standards for, 215, 223
- Bismuth caesium nitrite solution, 445  
sodium by, 444
- Bismuth oxide, iron in, 287
- Block comparator, 14
- Blood, ammonia in, 652  
arsenic in, 228, 231  
bismuth in, 216  
calcium in, 450, 451, 455, 458, 460, 461, 464  
calcium and magnesium in, 453  
carbon monoxide in, 113  
chlorides in, 534  
copper in, 160  
micro method for, 165, 166  
Folin-Wu precipitation of proteins in, 534  
hydrogen-ion concentration in, 696, 711  
iodine in, 559  
iron in, 291  
micro method for, 292, 299, 305  
magnesium in, 472, 476  
manganese in, 337  
nitrites in, 646  
perchloric acid oxidation of, 291  
peroxide oxidation of, 291  
phosphorus in, 487, 488, 490  
potassium in, 433  
sodium in, 443, 444  
sulfates in, 619, 620, 622  
thiocyanate in, 609  
trichloroacetic acid as protein precipitant for, 487, 488, 609, 619  
tungstic acid as protein precipitant for, 534  
uranium acetate as protein precipitant for, 622  
without ashing, iron in, 291  
zinc sulfate as protein precipitant for, 646
- Blood chemistry, colorimeter for, 47
- Blood filtrate, from Folin-Wu method of precipitation, 534  
from trichloroacetic acid precipitation, 487, 609, 619  
from tungstic acid precipitation, 534  
from uranium acetate precipitation, 622  
from zinc sulfate precipitation, 646
- Blood plasma. See plasma.
- Blood serum. See serum.
- Body fluids, lead in, 203
- Bomb decomposition of organic samples, 229
- Bone, arsenic in, 232  
bismuth in, 216  
copper in, 161  
lead in, 203
- Boric acid  
by curcumin, 526  
by turmeric paper, 527  
standards for, 527, 529
- Boron, by turmeric, 529  
standards for, 529
- Bottle for standard alkali solution, 673
- Bottles for comparison, 12
- Bougault's reagent, arsenic by, 243, 249
- Brass, aluminum in, 262  
antimony in, 251  
chromium in, 274
- Bread, hydrogen-ion concentration in, 691
- Brewery products, hydrogen-ion concentration in, 691
- Brilliance, definition of, 6
- Brines, iodides and iodates in, 567
- Bromides, by chlorine-water, 544  
by extraction as bromine, 547  
by fluorescein, 548  
by fuchsin, 546  
by gold chloride, 546  
by phenol red, 549  
by Schiff's reagent, 544  
in blood, 546  
in inorganic samples, 545  
in organic samples, 545  
separation from iodides, 549  
separation from reducing or oxidizing agents, 549



- Bromides, standards for, 544, 547, 550  
 Bromine. See bromides.  
 Bronze, aluminum in, 262  
 Brucine, nitrates by, 635  
 Buffers, acetate, 271, 273, 311, 548, 549  
     Clark and Lubs, 671  
     for carbon dioxide determination, 118  
     for high pH values, 682  
     for pH 1.0-2.2, 674  
     for pH 2.2-3.8, 675  
     for pH 2.2-8.0, 674, 677  
     for pH 2.2-10.0, series of, 671  
     for pH 4.0-6.2, 675  
     for pH 5.4-5.8, 271  
     for pH 5.8-8.0, 675  
     for pH 7, 671  
     for pH 7.8-10.0, 676  
     for pH 9.2-11.0, 677  
     for pH 11.0-12.0, 676  
     manipulation of, 674  
     principle of, 666  
 Cacothelin, 258  
     tin by, 258  
 Cadmium, as the sulfide, 176  
     in organic material, 176  
     standard for, 177  
 Calcium, as nickel nitrite complex by antipyrine, 462  
     as oleate, 452  
     as oxalate, 458, 463, 465  
     as pierolonate, 466  
     as ricinoleate, 451  
     as soap, 449  
     as stearate, 450  
     as tungstate by titanous chloride, 461  
     by alizarin, 465  
     by phenolic properties of hydroxyquinoline complex, 459  
     by reduction of phosphate with 1,2,4-aminonaphthol sulfonic acid, 455  
     by reduction of phosphate with hydroquinone, 454  
     by reduction of phosphate with stannous chloride, 456  
         micro method for, 457  
     by sodium sulforicinoleate, 453  
     by turbidity as oxalate, 463, 465  
     in blood, 450, 451, 455, 458, 460, 461, 464  
     in feces, 449, 450, 451  
     in milk, 450, 451  
     in organic samples, 450  
     in serum, 455, 458, 461  
     in urine, 449, 450, 451, 464  
     in water, 459  
     standards for, 451, 452, 454, 455, 456, 458, 459, 461, 462, 463, 464, 466  
 Calcium and magnesium as ferrocyanide, 453  
     in blood, 453  
     in tissue, 453  
     in water, 453  
     standard for, 454  
 Calcium phosphate, lead in, 190  
 Calculation of results, 97  
     for balancing method, 98  
     for dilution method, 97  
     for duplication method, 98  
     for series of standards method, 97  
 Calibration of color glasses, 32  
 Calibration of pyrotannic detector, 113  
 Camera, color, 36  
 Campbell-Hurley colorimeter, 39, 40  
 Cane juice, phosphorus in, 493  
 Carbon, 101  
     in iron. See iron.  
     in organic matter  
         by conversion to carbon dioxide, 104  
         apparatus for decomposition of, 105  
     in steel. See steel.  
 Carbonates, fluorides in, 585  
 Carbon bisulfide, as copper xanthate, 602  
     by copper and diethylamine, 603  
     by evolution as hydrogen sulfide, 602  
     standard for, 603  
 Carbon dioxide, absorbent solution for, 120, 122  
     apparatus for comparison in atmosphere of, 42  
     buffers for, in determination of, 118  
     by determination of pH, 119  
         calculations, 120  
         sources of error, 120  
     by the sodium salt of phenolphthalein, 121

- Carbon dioxide, by the sodium salt of phenolphthalein, absorbent solution for, 122  
apparatus for, 123  
carbon in organic matter by conversion to, 104  
carbon in steel by conversion to, 104  
in gases, 122  
in gases, apparatus for estimation of, 106  
in solids and liquids, 122  
standards for, 118, 121  
traces of, absorbent solution for, 120  
comparison solution for, 121
- Carbon disulfide. See carbon bisulfide.
- Carbon monoxide, by ammoniacal silver nitrate solution, 115  
by hemoglobin and pyrogallie and tannic acids, 112  
by hoolamite, 108  
detector, 108, 109, 110  
care of, 110  
by palladium chloride, 110  
by sulfuric acid and iodine pentoxide, 108  
in air, 112, 116  
in blood, 113  
in ethylene, 112, 113, 115  
in manholes and confined spaces, 109  
standards for, 110, 111, 112, 114, 115
- Carius oxidation of organic samples, 228
- Cast iron. See iron.
- Cells, hydrogen-ion concentration in, 696
- Cement, sulfates in, 613  
sulfur in, 613
- Cement, Portland. See Portland cement.
- Cerebrospinal fluid, hydrogen-ion concentration in, 696  
thiocyanate in, 610
- Cerium, by gallic acid, 366  
standard for, 366
- Chalybeate preparations, iron in, 289
- Chemicals present other than the test substance, error from, 82
- Chloramine, by Nessler's reagent, 542  
by o-tolidine, 543
- Chlorates, by ammonium thiocyanate, 589  
by aniline hydrochloride, 588  
perchlorates in, 591
- Chlorides, as silver chloride, 531  
by excess silver, 533  
by silver chromate, indirectly, 534  
as iodide, 536  
in blood, 534  
in phosphoric acid, 531  
in urine, 533  
standards for, 532  
turbidimetrically, 534
- Chlorine, by benzidine, 541  
by dimethyl-*p*-phenylenediamine, 542  
by starch-iodide solution, 542  
by o-tolidine, 538  
in air, 539  
in sewage, 539  
in water, 539  
standards for, 540, 541, 542
- Chromate. See chromium.  
by diphenylcarbazide, 624
- Chromate-cobalt-copper series of artificial standards, 67, 68
- Chromate-permanganate series of artificial standards, 68, 69
- Chromic ion. See chromium.
- Chromium, 274  
as chromate, 274  
as chromic ion, 277  
as dichromate, 276  
by after chroming dyed wool, 280  
by diphenylcarbazide, 279  
by disodium-1,8-dihydroxynaphthalene-3,6-disulfonate, 278  
in brass, 274  
in fabric, 275  
in iron, 274  
in ores, 275  
in plant ash, 279  
in rocks, 275  
in soils, 275  
in steel, 274, 278  
in tanning liquors, 278  
in tumors, 280  
separation from manganese and iron, 335  
standards for, 276, 277, 279, 281, 537
- Chromometer, Saybolt Universal, 28
- Cinchonine potassium iodide reagent, 219  
bismuth by, 218

- Citric acid and ammonium molybdate,  
    oxidizing power by, 138  
Citric acid extraction of soil, 491  
Clay, iron in, 285  
Coal, arsenic in, 238  
    sulfates in, 613  
    sulfur in, 613  
Cobalt, as chloride, 321  
    as cobaltamine, 324  
    as sulfate, 330  
    as sulfide, 330  
    by ammonium thiocyanate, 327  
    by arsenophosphotungstic acid, 328  
    by cobalt-cysteine complex, 329  
    by dimethylglyoxime, 327  
    by hydrogen peroxide, 330  
    by  $\alpha$ -nitroso- $\beta$ -naphthol, 322  
    by potassium ferrieyanide, 326  
    extraction with amyl alcohol, 328  
    in concentrated hydrochloric acid, 321  
    in steel, 325  
    separation from copper, 323  
    separation from copper and iron, 322  
    separation from manganese, 323  
    separation from nickel, 323  
    standards for, 322, 324, 326, 329  
Cobaltamine, 326  
    cobalt as, 324  
Cobalt-chromate-copper series of artificial  
    standards, 67, 68  
Cobalt-cysteine complex, 329  
Cobalt-iron-copper series of artificial  
    standards, 67, 68  
Cocaine-molybdate reagent, 242  
    arsenic by, 241  
Cocaine-strychnine reagent, 242  
    arsenic by, 241  
Collecting carbon dioxide over the sodium  
    salt of phenolphthalein, apparatus  
    for, 123  
Colloidal particles, error from variation  
    in size of, 88  
Color, definition of, 6  
    intensity, 8  
    quality of, 7  
    recording, systems of, 72  
Color camera, 36  
Color glasses, 32  
    calibration of, 31  
    standard, 32  
    variation in, 33  
Color plates, 31  
Color recording, systems of, 72  
Colored solutions, hydrogen-ion concen-  
    tration in, 715  
Colorimeter, Autenrieth-Koenigsberger,  
    54  
    Bader, 30  
    Campbell-Hurley, 39, 40  
    capillary for pH, 714  
    dilution, 36  
    Duboseq, 32, 43, 44, 45  
    for direct reading of ratio of sample to  
        standard, 47, 48  
    for nephelometric use, 49, 50  
    Hellige, 23  
    hydrogen-ion, 47, 49  
    Kennicott-Sargent, 39  
    micro Duboseq, 51  
    modified Campbell-Hurley, 41, 42  
    modified Duboseq, for pH, 702, 703  
    petroleum, 28  
    photoelectric, 57  
    prism type, 56  
    Rosenheim-Schuster, 20  
    Schreiner, 42  
    S. D. C., 17  
    special types, 61  
    Stammer, 28, 51, 52  
    Tag-Robinson, 29  
    Union, 30  
    Universal, 51  
    Vim-Sheftel, 47  
    wedge type, 53, 55  
    Wesson, 21, 63  
    with a cell for standard at constant  
        depth, 49  
    with artificial illumination, 50  
    with three cells, 49  
    Wulff, for pH, 716  
    Yoe photoelectric, 58  
Colorimetry, trichromatic, 27  
    without standards, 27  
Columbium, by reduction of the fluoride,  
    401  
    in tantalite, 401

- Columbium, standard for, 401  
Combined carbon, in iron, 103  
Comparator, Cooledge, 16  
    for pH measurement, 11, 685, 686  
    Hellige, 23, 24  
    capillary, 686  
    roulette, 15  
    sliding, 16, 685  
Comparison of artificial liquid standards, 69  
Comparison with color plates, 31  
Concentrated hydrochloric acid. See hydrochloric acid  
Conversion of Lovibond to Stammer readings, 29  
Cooledge comparator, 16  
Copper, as the bromide, 155  
    as the sulfide, 150  
    antimony in, 251  
    arsenic in, 230  
    bismuth in, 213, 214, 224  
    by ammonia, 143  
    by *m*-benzaminosemicarbazide, 166  
    by benzidine, 162  
    by comparison of the color of zinc mercurithiocyanate precipitates, 173  
    by dimethylglyoxime, 163  
    by diphenylthiocarbazone, 168  
        reagents for, 168  
    by direct green B, 175  
    by dithiooxamide, 169  
    by  $\beta$ -naphthol, 172  
    by piperidinium piperidylthioformate, 171  
    by potassium cyanide and guaiacum, 157  
    by potassium ethyl xanthate, 152  
    by potassium ferrocyanide, 147  
        precautions for, 150  
    by potassium iodide and starch, 156  
    by pyridine and thiocyanate, 159  
    by reduced phenolphthalein, 175  
    by salicylic acid, 146  
    by sodium arsenite, 174  
    by sodium diethyldithiocarbamate, 164  
    by urobilin, 172  
    electrolytic, lead in, 188  
    extraction of, with diphenylthiocarbazone in chloroform, 169  
    in aqueous humor, 161  
    in biological materials, 160, 161  
    in blood, 160, 165, 166  
    in bones, 161  
    in concentrated hydrochloric acid, 145  
    in food, 149, 164, 171  
    in gelatine, 148  
    in inorganic materials, 153  
    in iron and steel, 143  
    in lead carbonate and pig lead, 148  
    in legumes, 159  
    in medicinal iron preparations, 170  
    in milk, 161  
    in organic materials, 153, 160, 169, 171  
    in red lead, 149  
    in rubberized fabrics, 144  
    in slag, 144  
    in solutions containing iron, 151  
    in tailings, 144  
    in tanning extracts, 151  
    in tissue, 174  
    in vegetables, 144, 149  
    in water, 150, 151  
    in water in the presence of lead, 159  
    in wine, 161  
    lead in, 189  
    standards for, 145, 146, 150, 154, 156, 157, 162, 163, 167, 173  
Copper-cobalt-chromate series of artificial standards, 67, 68  
Copper-cobalt-iron series of artificial standards, 67, 68  
Copper foil, removal of antimony on, 251  
Copper ore, bismuth in, 213  
Corpuscles, phosphorus in, 487, 488, 489  
Correction curve for nephelometer, 92  
Crackers, hydrogen-ion concentration in, 693  
Cream, magnesium in, 476  
    hydrogen-ion concentration in, 690  
Cream of tartar, lead in, 196  
Cryogenine Lumière, copper by, 167  
Cupferron, aluminum by, 269  
Cuprous chloride, oxygen by, 130, 131  
Curcumin, beryllium by, 398  
    boric acid by, 526

- Curcumin, magnesium by, 477
- Cyanides**, 125
- as ammonia by Nessler's reagent, 127
  - as ferric thiocyanate, 125
  - as Prussian blue, 126
  - by picric acid, 129
  - gold in, 402, 403, 404
  - in inorganic samples, 125
  - in organic samples, 125
  - standards for, 126, 127
- Detectors** for carbon monoxide, hoolomite, 108, 109, 110, 111
- palladium chloride, 110, 111
  - pyrotannic, 115
- Development of nephelometer, 91
- Dichromatic solutions, apparatus for measuring color of, 47, 49
- error from comparing, 88
- Dichromatism. See dichromatic solutions
- Dichromate. See chromium
- Diethyldithiocarbamate, sodium, copper by, 164
- Dihydroxymaleic acid, titanium by, 365
- Dilution errors, 80
- Dilution method, 1, 35
- accuracy of, 74
  - calculation for, 97
- Dimethylaniline, nitrites by, 647
- Dimethylglyoxime, cobalt by, 327
- copper by, 163
  - iron by, 308
  - nickel by, 314
- Dimethyl-*p*-phenylenediamine, chlorine by, 542
- Dimethyl- $\alpha$ -naphthylamine, nitrites by, 645
- Diphenylamine, 372
- nitrate by, 637, 638
  - vanadium by, 372
- Diphenylamine sulfonic acid, nitrates by, 640
- Diphenylbenzidine, nitrates by, 638
- Diphenylcarbazide, chromium by, 279
- s*-Diphenylcarbazide, lead by, 198, 199
- Diphenylthiocarbazone, 168, 351
- copper by, 168
  - lead by, 202
  - zinc by, 350
- $\alpha$ ,  $\alpha'$ -Dipyridyl, 311
- iron by, 310
- Direct green B, copper by, 175
- Disodium-1, 8-dihydroxynaphthalene-3, 6-disulfonate, chromium by, 278
- Dissolved oxygen, 130, 131, 136, 137
- apparatus for, 130, 131
- Distillation, of arsenic, 237, 240, 248
- of arsenic and selenium, 233
  - of fluorine, 578, 579
  - of iodine, 561
  - of phosphorus, 495
  - of sulfides, 599
- Dithiooxamide, copper by, 169
- Dithizone. See diphenylthiocarbazone
- Dry ashing of organic samples, 235, 262, 496
- Duboseq colorimeter, 43, 44, 45
- micro, 51
- Duplication method, 2, 36
- accuracy of, 74
  - calculation for, 98
- Dyed wool, chromium by, 280
- Dyes, food, arsenic in, 232
- Eggertz tubes**, 13
- Eggs, iodine in, 558
- Egg yolk, iron in, 311
- Electrolytic copper. See copper
- Electrolytic Gutzeit method, 239
- apparatus for, 239, 240
  - modified, 240, 241
- Enamel, fluorides in, 573
- Eosin, potassium by, 438
- sodium by, 438
- Equipment, nephelometric, 91
- Eriochrome cyanine, aluminum by, 271
- Error, sources of. See sources of error
- Errors of artificial standards, 69
- Erythrosine, arsenic in, 233
- Ether-amyl alcohol, extraction of iron by, 297
- Ether solutions, apparatus for comparison of, 41
- Ethylene, carbon monoxide in, 112, 113, 115

- Ethylene, separation from carbon monoxide, 112  
apparatus for, 113
- Evolution of gases. See distillation
- Fabric.** See textiles
- Fat, iodine in, 557
- Feces, bismuth in, 216  
calcium in, 449, 450, 451  
iron in, 293, 299  
phosphorus in, 493  
sodium in, 445  
sulfides in, 594  
thiocyanate in, 609  
zinc in, 351
- Feed, ammonia in, 653  
phosphorus in, 493
- Ferric iron. See iron
- Ferric ferrithiocyanate, iron as, 284
- Ferric thiocyanate, as reagent for cyanides, 125
- Ferric thiocyanate reagent, 459  
calcium by, as oxalate, 458  
fluorides by bleaching of, 574  
magnesium by, as phosphate, 469
- Ferrocyanide, calcium and magnesium as, 453  
magnesium by, 475
- Ferromolybdenum, molybdenum in, 380
- Ferrosilicon, titanium in, 359
- Ferrous ammonium sulfate, hydrogen peroxide by, 139
- Ferrous iron. See iron  
completely reduced, 139
- Fertilizer, phosphorus in, 493
- Filaments, tungsten in, 376
- Filings, platinum in, 415
- Films, tungsten in, 376
- Fluorescein, bromides by, 548  
ozone by, 142
- Fluorides, by alizarin sodium sulfonate and zirconium nitrate, 580  
by bleaching oxidized titanium, 571  
by bleaching ferric acetylacetone, 576  
by bleaching ferric thiocyanate, 574  
chart for, 576  
by estimation of lead sulfide, 584  
by zirconium nitrate and 1,2,5,8-tetrahydroxyanthraquinone, 583  
by zirconium oxychloride and 1,2,4-trihydroxyanthraquinone, 582  
evolution of, 578, 579  
in animal matter, 585  
in basic slag, 572  
in carbonates, 585  
in enamel, 573  
in rock, 572  
in sea water, 582  
in silicates, 585  
in soil, 574  
in vegetable matter, 585  
in water, 574, 581, 584, 585  
correction chart for, 575  
standards for, 573, 580, 587  
traces of, 585  
separation from interfering substances, 585, 586  
volatilization as silicon fluoride, 577  
apparatus for, 578, 579
- Foliage, sprayed  
arsenic in, 232
- Folin's phenol reagent. See phosphomolybdotungstic acid
- Folin-Wu blood filtrate. See blood filtrate
- Food. See foodstuffs
- Food dyes, arsenic in, 232
- Foodstuffs, copper in, 149, 164, 171  
iodine in, 553, 554  
apparatus for, 553, 555  
sulfates in, 612  
sulfides in, 594  
sulfur in, 612
- Forage, iron in, 294
- Formaldoxime reagent, 319  
nickel by, 319
- Fruit peelings, arsenic in, 230
- Fuchsin, 546  
bromides by, 546
- Furfural, sulfates by, 622
- Gall, sodium in, 445
- Gallic acid, cerium by, 366  
titanium by, 365
- Gaseous oxygen, 133, 136, 138  
apparatus for, 134
- Gases, carbon bisulfide in, 602  
carbon dioxide in, 106, 122

- Gases, carbon dioxide, apparatus for, 106
  - oxygen in, 133, 138
  - apparatus for, 134
  - phosphorus in, 515
- Gastric contents, hydrogen-ion concentration in, 695
- Gastric juice, thiocyanate in, 610
- Gelatine, arsenic in, 230
  - copper in, 148
  - hydrogen-ion concentration, 693
  - sulfides in, 594
- Gelatine solution. See gelatine
- Gillespie comparator, 12
- Glass, titanium in, 358
- Glasses. See color glasses
- Glass standards, 32
  - for Duboseq colorimeter, 32
  - for hydrogen-ion determination, 686
- Glue, hydrogen-ion concentration in, 693
- Gold, as colloidal gold, 402
  - by mercurous chloride, 407
  - by *o*-tolidine, 408
  - in cyanides, 402, 403, 404
  - in ores poor in gold, 404
  - in tissue, 404
  - separation from platinum and palladium, 421
  - standards for, 407
- Grain, arsenic in, 232
  - iron in, 294, 311
  - thallium in, 209
- Guinea green B, arsenic in, 232
- Gum arabic as protective colloid, 649
- Gutzeit method, apparatus for, 236, 237
  - arsenic by, 225
  - electrolytic. See electrolytic Gutzeit method
  - modified, by silver nitrate, 247, 248
  - modified electrolytic, 240, 241
  - test paper for, 236
- Hehner** cylinders, 30, 33, 39
- Hellige capillary comparator, 686
- Hellige colorimeter, 23
- Hematin, lead by, 205
- Hematoxylin, aluminum by, 266
- Hemoglobin and pyrogallol and tannic acids as reagent for carbon monoxide, 112, 113
- Hoolomite, 108
- Hops, arsenic in, 232
- Hue, definition of, 6
- Hydrochloric acid, concentrated, cobalt in, 321
  - copper in, 145
  - iron in, 307
  - rhodium in, 424
- Hydrochloric acid extraction of soil, 491
- Hydrocyanic acid, apparatus for hydrolysis of, 128
  - as ammonia, 127
- Hydrogen-ion buffers. See buffers
- Hydrogen-ion color comparator, 16
- Hydrogen-ion concentration
  - absorption curves, 661
  - by Pulfrich photometer, 711
  - calculation of results, 704
  - carbon dioxide estimation by, 119
  - colorimeter for, 47, 49
  - colorimetric principles for, 666
  - comparison on a spot plate, 717
  - comparison with standard buffers, 697
  - comparison with test strips, 717
  - correction factors for, in milk and whey, 692
  - estimation without buffers, 697
- Gillespie-Hatfield indicators for, 698
- glass standards for, 686
- in acetone-water mixtures, 688
- in biological samples, 695
- in blood, 696, 711
- in bread, 691
- in brewery products, 691
- in cells, 696
- in cerebrospinal fluid, 696
- in clear liquids, 687
- in colored solutions, 715
- in crackers, 693
- in cream, 690
- in gastric contents, 695
- in gelatine solution, 693
- in glue, 693
- in meat extract, 693
- in milk, 689, 690, 692
  - condensed, 691
  - evaporated, 691

## Hydrogen-ion concentration

- in milk, powdered, 691
- in molasses, 688
- in nutrient agar, 694
- in plasma, 696, 711
- in sea water, 687
- in serum, 696, 711
- in soap solution, 693
- in soil, 688
- in sugar solutions, 688
- in sulfated fatty alcohols, 693
- in sulfonated oil, 688
- in textile assistants, 694
- in tissues, 696
- in unbuffered solutions, 694
- in urine, 695
- in water, 687
- in whey, 690, 692
- interpretation, 664
- interrelation of pH,  $C_H$  and  $C_{OH}$ , 665
- methods, 697
- principle of buffer solutions, 666
- readings for colorimeter factor, at 10 mm., 704
- readings for colorimeter factor, at 15 mm., 705
- readings for colorimeter factor, at 20 mm., 705
- readings for colorimeter, standard at 15 mm., 706, 707
- readings for colorimeter, standard at 10 mm., 708
- readings for colorimeter, standard at 20 mm., 709, 710
- sources of error, 667
  - acid contamination, 667
  - alkaline contamination, 667
  - dilution, 667
  - filtration, 667
  - indicator, 669
  - miscellaneous, 670
  - protein, 669
  - salt, 667, 668
  - temperature, 670
- standards for, bromocresol purple, 700
- bromophenol blue, 699
- bromothymol blue, 700
- cresol red, 701
- methyl red, 700

phenol red, 701

thymol blue, 701

standard tubes for, 697, 699

Hydrogen-ion indicators. See indicators

Hydrogen peroxide, by ammonium molybdate, 140

by destruction of nitrite, 140

by liberation of iodine, 141

by oxidation of ferrous iron, 139

by oxidation of titanium sulfate, 141

cobalt by, 330

molybdenum by, 380

standards for, 139, 140

titanium by, 355

vanadium by, 367, 370

Hydrogen sulfide, as colloidal lead sulfide, 596

as sulfate, 601

evolution of, 598, 599

in water, 596

Hydrolysis, of hydrocyanic acid, 127

apparatus for, 128

Hydroquinone, calcium by, as phosphate, 454

magnesium by, as phosphate, 468

phosphorus by, as phosphomolybdate, 504

tungsten by, 378

1,2,5,8-Hydroxyanthraquinone. See quin-alizarine

o-Hydroxybenzoic acid, uranium by, 394

Hydroxylamine, selenium by, 606

Hydroxymethylantraquinone, aluminum by, 271

8-Hydroxyquinoline, 268, 475

aluminum by, 267

calcium by, 459

iron by, 311

magnesium by, 471, 473, 474

o-Hydroxyquinoline, bismuth by, 221

## Illumination, 6

errors from variable, 77

Importance of errors, 89

Impurity of reagents, as source of error, 89

Indicators, 677

color changes of, 683



- Indicators, monochromatic, of Michaelis, 678  
of Clark & Lubbs, Cohen, and La Motte Chemical Products Co., 681  
of Sorensen, 679, 680  
pH of, 682, 683, 684  
universal indicator, 685
- Indigo carmine, oxygen by, 137
- Interrelation of pH,  $C_H$  and  $C_{OH}$ , 665
- Inorganic materials, arsenic in, 227  
bismuth in, 213  
bromides in, 545  
copper in, 153  
cyanides in, 125  
lead in, 188
- Iodates and Iodides. See iodides and iodates
- Iodide, by mercurous chloride, 568  
by starch-iodine reaction, 569  
in iodized salt, 569  
standard for, 570
- Iodides and iodates, by *o*-tolidine, 567  
in water, 567  
in salt brines, 567
- Iodine, as silver iodide, 563  
by bromine-water, 566  
by carbon bisulfide extraction, 565  
by distillation, 560  
apparatus for, 561  
by palladous chloride, 564  
by solvent extraction, 551  
in blood, 559  
in dry milk, 558  
in eggs, 558  
in fat, 557  
in foodstuffs, 553, 554  
apparatus for, 553, 555  
in nuts, 558  
in rock, 560  
in seeds, 558  
in soil, 560  
in thyroid, 556  
in urine, 558, 559, 563  
in vegetables, 553  
apparatus for, 553  
in water, 552  
standards for, 563, 567, 568, 570
- Iodine pentoxide and sulfuric acid as carbon monoxide detector, 108
- Iodized salt, iodide in, 569
- Iodobismuthate of quinine, bismuth as, 221
- 7-Iodo-8-hydroxyquinoline-5-sulfonic acid  
iron by, 302
- Iridium, by benzidine, 425  
in ammonium chlorplatinate, 425  
in hydrochloric acid, 426  
in platinum, 425  
separation from other metals, 417
- Iron, as the chloride, 307  
as the sulfide, 306  
by acetylacetone, 303  
by alloxantin, 310  
by dimethylglyoxime, 308  
by  $\alpha$ ,  $\alpha'$ -dipyridyl, 310  
by 8-hydroxyquinoline, 311  
by 7-iodo-8-hydroxyquinoline-5-sulfonic acid, 302  
by mercaptoacetic acid, 298  
by potassium ferrocyanide, 304  
by pyrimidone, 309  
by pyrocatechol, 309  
by salicylic acid, 301  
by salicysulfonic acid, 302  
by thiocyanate, 283  
reagent for, 295  
by thioglycolic acid, 298  
by thiosalicylic acid, 300  
carbon in, 103  
cast, titanium in, 358  
chromium in, 274  
copper in, 143  
ferric. See also iron  
by thiosalicylic acid, 300  
in water, 288  
in wine, 294  
standards for, 297, 298, 309
- ferrous, as the sulfide, 306  
by potassium ferricyanide, 305  
by thiosalicylic acid, 300  
in water, 289, 306  
in wine, 294  
standards for, 306, 307
- hematin, 311
- in alloys, 301, 307  
in battery acid, 287

- Iron, in biological material, 312  
     micro method for, 312  
 in bismuth, 287  
 in bismuth oxide, 287  
 in blood, 291  
     micro method for, 292, 299, 305  
 in chalybeate preparations, 289  
 in clay, 285  
 in concentrated hydrochloric acid, 307  
 in egg yolk, 311  
 in feces, 293, 299  
 in forage, 294  
 in grain, 294, 311  
 in lead, 286  
 in lead compounds, 286  
 in metals, 301  
 in milk, 290, 298  
 in organic samples, 304  
 in oxides, 308  
 in plants, 295  
 in plasma, 292  
 in Portland cement, 285  
 in salts, 308  
 in sea water, 289  
 in silica, 285  
 in silicates, 285  
 in soil extracts, 301  
 in solution, 308  
 in tanning extracts, 294  
 in textiles, 295  
 in tissue, 293  
 in urine, 290, 299  
 in water, 288, 289  
 in wine, 294  
 in yeast, 311  
 in zinc oxide, 287  
 inorganically combined in water, 289  
 molybdenum in, 385  
 nonhematin, 311  
 phosphorus in, 484, 495, 496, 513  
 separation from chromium, 335  
 sulfides in, 594  
 titanium in, 360  
 tungsten in, 377  
 zinc in, 346
- Iron-cobalt-copper series of artificial standards, 67, 68
- Iron preparations. See medicinal iron preparations
- Insect tissue, arsenic in, 239, 240
- Ives tint photometer, 25
- Jars, Nessler, 14  
     standard, 13
- Julian tubes, 13
- Kennicott-Sargent colorimeter, 39
- Kober nephelometer, 92
- Law, Beer's, 3  
     Ostwald's, 96
- Lead, as the chromate, 196, 197  
     as the chromate by *s*-diphenylcarbazide, 199  
     as the molybdate, 204  
     as the sulfide, 186, 193, 196  
     bismuth in, 214  
     by hematin, 205  
     by *s*-diphenylcarbazide, 198, 199  
     by diphenylthiocarbazone, 202  
     by dithizone, 202  
     by sodium bisulfite, 206  
     by tetramethyldiaminodiphenylmethane, 200  
     in aqueous solutions, 192  
     in baking powder, 186  
     in body fluids, 203  
     in bones, 203  
     in calcium phosphate, 190  
     in commercial phosphoric acid, 189  
     in cream of tartar, 196  
     in electrolytic copper, 188  
     in inorganic samples, 188  
     in lead peroxide, 205  
     in medicinal iron preparations, 188  
     in metals, 203  
     in organic samples, 186, 187, 188  
     in organs, 203  
     in samples not containing organic matter, iron, aluminum, or copper, 188  
     in solutions high in iron, 192  
     in spinal fluid, 203  
     in tissue, 187, 203  
     in urine, 190, 191, 196, 203  
     in water, 192, 193

- Lead, iron in, 286  
standards for, 193, 197, 198, 205
- Lead alloys, aluminum in, 262  
bismuth in, 214
- Lead carbonate, copper in, 148  
iron in, 286
- Lead compounds, iron in, 286
- Lead dioxide, by oxidation of aniline, 206
- Lead ore, bismuth in, 213
- Lead oxides, iron in, 286  
lead in, 205
- Lead, pig, copper in, 148  
iron in, 286
- Lead, red, copper in, 149
- Legumes, copper in, 159
- Light, standard, 7
- Light source, 6  
errors from variable, 77
- Light green S F yellowish, arsenic in, 232
- Liquid standards, artificial, natural. See standards
- Lithium, as stearate, 447
- Lovibond tintometer, 18, 19, 34  
calibration of, 32, 33  
conversion of readings to Stammer readings, 29  
glasses for, 32  
variation in, 33
- Magnesium**, as alizarinate, 478  
as 8-hydroxyquinoline complex, by conversion to dye, 474  
by decolorization of, 474  
by iron, 473  
by phenolic properties of, 471  
as oleate, 478  
as phosphate, 479  
by conversion to phosphomolybdate, 470  
by ferrie thiocyanate, 469  
by hydroquinone, 468  
by ammonium ferrocyanide, 475  
by curcumin, 477  
by titan yellow, 476  
in blood, 472, 476  
in cream, 476  
in meat, 476  
in meat extract, 476  
in organic samples, 469  
in serum, 472, 476  
in soil extracts, 476, 477  
in urine, 468, 472, 476  
standards for, 470, 471, 473, 477, 478
- Magnesium and calcium. See calcium and magnesium
- Magnetite, vanadium in, 373
- Malt, arsenic in, 232
- Manganese, as permanganate, 332  
by benzidine, 343  
by tetramethyldiaminodiphenylmethane, 342  
by *o*-tolidine, 341  
in alloy steels, 334  
in blood, 337  
in minerals, 335  
in ores, 336  
in pharmaceutical preparations, 336  
in phosphoric acid, 336  
in pig iron, 334  
in rocks, 335  
in soil, 335  
in steel, 334  
in textiles, 338  
in tissue, 337  
in urine, 338  
in vegetable matter, 337  
in water, 335, 342  
separation from chromium, 335  
standards for, 341, 343, 344
- Matte, tungsten in, 377
- Meat, magnesium in, 476  
nitrates in, 635
- Meat extract, hydrogen-ion concentration in, 693  
magnesium in, 476
- Mechanical errors, 75
- Medicinal iron preparations, copper in, 170  
lead in, 188
- Mercaptoacetic acid, iron by, 298
- Mercuric bromide paper, for arsenic, 236
- Mercurithiocyanate solution, 173
- Mercurous chloride, arsenic by, 250  
gold by, 407  
iodide by, 568

- Mercurous chloride, selenium by, 606  
tellurium by, 607
- Mercury, as sulfide, 178  
by molybdotungstic acid, 183  
by phosphomolybdotungstic acid, 183  
by phosphotungstic acid, 183  
by potassium diphenylcarbazone, 180  
by potassium iodide and ammonium hydroxide, 182  
by selenium sulfide, 184  
by strychnine sulfate and potassium iodide, 185  
distillation in chlorine, 181  
distillation of, 179  
in air, 179  
in fabrie, 182  
in organic samples, 178  
removal by copper wire, 178  
standards for, 180, 181
- Metallic copper. See copper
- Metals, iron in, 301, 307  
lead in, 203  
platinum in, 416
- Metaphenylenediamine, nitrites by, 647
- Method, definition of, balancing, 1  
dilution, 1  
duplication, 2  
series of standards, 1
- Methods, colorimetric general  
balancing, 1, 38, 74, 98  
comparison with a series of standards,  
1, 10, 74, 97  
comparison with solid standards, 19  
definition, 1  
development of, 2  
dilution, 1, 35, 74, 97  
duplication, 2, 36, 74, 98  
extent of use, 2  
limitations, 73, 74  
origin of, 8  
sources of error, 74
- Micro Duboscq colorimeter, 51
- Milk, ammonia in, 652  
calcium in, 450, 451  
condensed, hydrogen-ion concentration  
in, 691  
copper in, 161  
evaporated, hydrogen-ion concentration  
in, 691  
hydrogen-ion concentration in, 689,  
690, 692  
iron in, 290, 298  
phosphorus in, 490  
powdered, hydrogen-ion concentration  
in, 691  
iodine in, 558  
sodium in, 445  
sulfates in, 622
- Minerals, manganese in, 335  
phosphorus in, 484, 491  
silicate, titanium in, 356  
tungsten in, 375  
vanadium in, 370, 371
- Mineral waters, sulfides in, 594
- Mixed gases. See gases
- Modified Campbell-Hurley colorimeters,  
41, 42
- Molasses, hydrogen-ion concentration in,  
688
- Molybdates, molybdenum in, 380
- Molybdenum, as the sulfide, 381  
by phenylhydrazine, 389  
by hydrogen peroxide, 380  
by potassium thiocyanate, 383  
by potassium xanthate, 383  
by sodium thiosulfate, 392  
by tannic acid, 389  
by tungstic acid reagent, 390  
extraction with ether, 386  
extraction with cyclohexanol, 386  
in alkali molybdates, 380  
in ammonium molybdate, 380  
in ferromolybdenum, 380  
in manganese-iron ore, 389  
in molybdic acid, 380  
in plants, 385  
in soil, 386  
in steel, 384, 385, 388, 391, 392  
in tungsten, 384  
in tungsten and molybdenum deposits,  
380  
wire, 381  
in tungstic acid, 381, 384  
separation from manganese and iron,  
389

- Molybdenum, standards for, 381, 382, 387, 388, 390
- Molybdenum blue, arsenic by formation of, 244
- Molybdenum and tungsten, molybdenum in, 380, 381
- Molybdic acid, molybdenum in, 380
- Molybdotungstic acid, mercury by, 183
- Monoxide, carbon. See carbon monoxide
- Naphthol**, arsenic in, 232
- $\alpha$ -Naphthylamine and  $\beta$ -naphthylamine-6,8-disulfonic acid, nitrites by, 646
- $\alpha$ -Naphthylamine and sulfanilic acid, nitrites by, 644
- $\alpha$ -Naphthylamine and tartaric acid, nitrites by, 645
- Nephelometer, applications for, 93
- correction curve for, 92
- development of, 91
- for series of standards, 10
- Kober, 92
- range of usefulness of, 93
- Nephelometric equipment, 91
- Nephelometry, general, 90
- applications of, 93
- Nessler's reagent, ammonia by, 649
- chloramine by, 542
- Folin-Wu modification, 654
- Frederick's modification, 654
- Jackson's modification, 653
- Treadwell modification, 654
- Winkler's modification, 654
- Nessler tubes, 13, 14, 38, 42
- Nickel, by dimethylglyoxime, 314
- by formaldoxime reagent, 319
- by potassium dithiooxalate, 317
- by potassium thiocarbonate, 313
- extraction with ether, 316
- in alloys, 315
- in biological samples, 315 \*
- in cobalt salts, 315
- in nickel steel, 315
- standards for, 314, 316
- Nickel ammonium sulfate, as standard for nickel, 314
- Nickel nitrite reagent, 463
- calcium by, 462
- Nitrates, by brucine, 635
- by diphenylamine, on a spot plate, 637
- in sulfuric acid, 638
- by diphenylamine sulfonic acid, 640
- by diphenylbenzidine, 638
- by estimation as picric acid, 642
- by phenoldisulfonic acid, 629
- by pyrogallol, 640
- by pyrogallol sulfonic acid, 640
- by reduction to ammonia, 642
- by strychnine and sulfuric acid, 636
- in meat, 635
- in organic samples, 632
- in oxides of nitrogen, 632
- in plant materials, 632
- in soil, 630, 631, 638, 641
- in sulfuric acid, 642
- in water, 629, 641
- standards for, 634, 637, 640
- Nitric acid, palladium by, 427
- Nitrites, by antipyrine, 648
- by conversion to nitrates, 648
- by dimethylaniline, 647
- by dimethyl- $\alpha$ -naphthylamine, 645
- by metaphenylenediamine, 647
- by  $\alpha$ -naphthylamine and  $\beta$ -naphthylamine-6, 8-disulfonic acid, 646
- by  $\alpha$ -naphthylamine and tartaric acid, 645
- by sulfanilic acid and  $\alpha$ -naphthylamine, 644
- by zinc iodide and starch solution, 648
- in blood, 646
- standards for, 645, 647, 648
- Nitrogen, albuminoid, 650, 651
- ammoniacal, 649, 650, 651
- apparatus for decomposition of organic matter, to determine, 105
- as ammonia, 649
- organic, 650, 651, 652
- Nitrosodimethylaniline, perchlorates by, 592
- $\alpha$ -Nitroso- $\beta$ -naphthol, 324
- cobalt by, 322
- Nuts, iodine in, 558
- Oils**, apparatus for determining color of, 21

- Oils, arsenic in, 236  
carbon bisulfide in, 602  
color of, 21  
sulfonated, hydrogen-ion concentration in, 688
- Operator's errors, 85
- Optical errors, 75
- Ores, bismuth in, 218  
chromium in, 275  
gold in, 404  
manganese in, 336  
platinum in, 413  
titanium in, 360  
tungsten in, 377  
uranium in, 395
- Organic samples, acid ashing, 497  
antimony in, 252  
arsenic in, 228, 229, 239  
bismuth in, 215, 223  
bomb decomposition of, 229  
bromides in, 545  
calcium in, 450  
carbon in, 104  
Carius oxidation of, 228  
copper in, 153, 154, 160, 169, 171  
cyanides in, 125  
dry ashing of, 235, 496  
fluorides in, 585  
iron in, 304  
lead in, 186, 187, 188  
magnesium in, 469  
mercury in, 178  
nitrates in, 632  
perchloric acid ashing, 498  
peroxide ashing, 499  
phosphorus in, 494  
potassium in, 430  
uranium in, 395  
wet ashing, 497  
zinc in, 348, 349, 350, 353
- Organs, lead in, 203  
sodium in, 445
- Orthophosphoric acid. See phosphorus
- Orthotolidine test, apparatus for, 30
- Osmium, separation from other metals, 416
- Ostwald's law, 96
- Oxides, iron in, 308
- Oxides of nitrogen, nitrates in, 632
- Oxidizing power of compounds, as oxygen, 138
- Oxygen, 130  
active, in oxidizing compounds, by citric acid and ammonium molybdate, 138  
dissolved, by adurol, 136  
by cuprous chloride, 130  
by indigo carmine, 137  
gaseous, by oxidation of nitric oxide, 138  
by pyrogallol, 136  
by the starch-iodine complex, 133  
standards for, 133, 135, 136, 137
- Oxygen absent, apparatus for comparison with, 42
- Ozone, by destruction of nitrite, 141  
by fluorescein, 142  
by liberation of iodine, 141  
standard for, 142
- Palladium, by mercurous chloride, 420  
by nitric acid, 427  
in platinum by potassium iodide, 428  
separation from gold and platinum, 421  
separation from other metals, 417  
standards for, 428
- Palladium chloride, as carbon monoxide detector, 110
- Paper, prepared, arsenious oxide, 597  
for pH determination, 717  
lead acetate, 599  
mercuric bromide, 236  
selenium sulfide, 184  
silver nitrate, 247  
thiocyanate, 589  
turmeric, 528
- Perchlorates, by methylene blue, 590  
by nitrosodimethylaniline, 592  
in chlorates, 591  
in plating baths, 590  
in solid salts, 590  
standard for, 592
- Perchloric acid ashing of samples, 498
- Permanganate-chromate series of artificial standards, 68, 69
- Peroxide ashing of samples, 499

- Petroleum distillates, sulfur in, 600  
pH. See hydrogen-ion concentration  
Pharmaceutical preparations, manganese in, 336  
Phenol reagent. See phosphomolybdotungstic acid reagent  
Phenol red, bromides by, 549  
Phenol and sodium hypochlorite, ammonia by, 658  
Phenoldisulfonic acid reagent, 633  
nitrates by, 629  
Phenolic acids, uranium by, 394  
Phenolphthalein, reduced, copper by, 176  
Phenolphthalein, sodium salt of, for determination of  $\text{CO}_2$ , 121  
apparatus for, 123  
Phenylhydrazine, molybdenum by, 389  
Phosphates. See phosphorus  
Phosphine, distillation of, 495  
phosphorus as, 494, 495  
Phosphomolybdate, phosphorus as, 480  
by reduction with aminonaphthol sulfonic acid, 505  
by reduction with benzidine, 507  
by reduction with hydroquinone, 504  
by reduction with hydrazine sulfate, 502  
by reduction with reduced molybdic acid, 506  
by reduction with stannous chloride, 503  
thallium as, 210  
Phosphomolybdotungstic acid reagent, 183, 461  
aluminum by, 270  
antimony by, 254  
bismuth by, 219  
calcium by, 459  
mercury by, 183  
zinc by, 352  
Phosphor-bronze, aluminum in, 262  
Phosphoric acid, arsenic in, 230  
chlorides in, 531  
lead in, 189  
manganese in, 336  
Phosphorus, acid-soluble, 488  
as magnesium ammonium phosphate, 483  
as phosphine, 494  
apparatus for, 495  
as phosphomolybdate, 480  
by reduction of, 486  
as phosphovanadomolybdate, 485  
as silver phosphate, 510  
as uranium acetate, 514  
by ammonium molybdate, 485  
by molybdenum sulfide, 484  
by quinine reagent, 514  
by silver nitrate, 516  
by strychnine reagent, 511, 513  
by uranium acetate and potassium ferrocyanide, 514  
in aluminum, 494  
in bile, 488  
in blood, 487, 488, 490  
in cane juice, 493  
in cast iron, 513  
in combustible gases, 515  
in corpuscles, 487, 488, 489  
in feces, 493  
in feed, 493  
in fertilizer, 493  
in iron, 484, 495, 496, 513  
in milk, 490  
in minerals, 484, 491  
in organic compounds, 494  
in plants, 493  
in plasma, 487, 488  
in sea water, 509  
in seeds, 489  
in serum, 487, 488, 513  
in soil extracts, 491, 492  
in steel, 484, 485, 495, 496  
in sugar, 493  
in tissue, 489  
in urine, 486, 490  
in water, 508  
in wine, 490, 491  
inorganic, 486, 487, 488, 490  
lipoid, 488, 489, 490, 515  
organic, 486, 490, 493  
orthophosphoric acid in phosphoric acid mixtures, 494  
standards for, 455, 456, 471, 482, 484, 485, 507, 508, 509, 522  
total, 486, 487, 491, 493

- Phosphorus pentoxide. See phosphorus  
Phosphorus and silica. See silica and phosphorus  
Phosphotungstic acid, mercury by, 183  
vanadium by, 373  
Photoelectric colorimeter, 57  
Photometer, Ives tint, 25  
Pulfrich, 26, 27  
hydrogen-ion concentration by, 711  
Photometry, 90, 93  
Pierolonate, calcium as, 466  
Pig iron, manganese in, 334  
titanium in, 359  
Pig lead, copper in, 148  
Piperidinium piperidylthioformate, copper by, 171  
Plant ash, chromium in, 279  
vanadium in, 373  
Plants, iron in, 295  
molybdenum in, 385  
nitrates in, 632  
phosphorus in, 493  
sodium in, 445  
Plasma, hydrogen-ion concentration in, 696, 711  
iron in, 292  
phosphorus in, 487, 488  
silica in, 524  
sodium in, 444  
Plating baths, perchlorates in, 590  
Platinum, as the iodide, 419  
sources of error, 419  
by mercurous chloride, 420  
by reduction to platinous chloride, 413  
in concentrates, 413  
in filings, 415  
in mixed platinum metals, 416  
in ores, 413  
in sands, 413  
in sweepings, 415  
iridium in, 425  
palladium in, 428  
separation by fire assaying, 413  
separation from gold and palladium, 421  
separation from iridium, 417  
separation from osmium, 416  
separation from palladium, 417  
separation from rhodium, 417  
separation from ruthenium, 416  
standards for, 418, 420  
Portland cement, iron in, 285  
Potassium, as chloroplatinate, by potassium iodide, 431  
by reduction with stannous chloride, 430  
as cobaltinitrite, 432  
as picrate, 437  
by eosin, 438  
by sodium cobaltinitrite, 432  
in blood, 433  
in blood serum, 434  
in organic samples, 430  
in soil, 430, 434  
in soil extracts, 430  
in solutions containing phosphates or iron, 434  
in water, 430  
separation from phosphates and iron, 434  
standards for, 431, 437  
Potassium cyanide and guaiacum, copper by, 157  
Potassium diphenylcarbazone, 181  
mercury by, 180  
Potassium dithiooxalate, 318  
nickel by, 317  
Potassium ethyl xanthate, copper by, 152  
Potassium ferricyanide, cobalt by, 326  
uranium by, 395  
zinc by, 348  
Potassium ferrocyanide, iron by, 304  
uranium by, 395  
zinc by, 348  
Potassium iodide, 156  
copper by, 156  
mercury by, 182, 185  
Potassium pyroantimonate, sodium by, 443  
Potassium thiocarbonate, nickel by, 313  
Potassium thiocyanate. See thiocyanate  
Potassium xanthate, molybdenum by, 383  
Prism type colorimeter, 56  
Protein precipitation in blood. See blood  
Prussian blue, cyanides as, 126  
Pulfrich photometer. See photometer



- Pyramidone, iron by, 309  
 Pyridine and iodide, antimony by, 253  
 Pyridine and thiocyanate, copper by, 159  
 Pyrites, arsenic in, 234  
     selenium in, 606  
 Pyrocatechol, iron by, 309  
 Pyrogallol, 641  
     nitrates by, 640  
 Pyrogallol sulfonic acid, 641  
     nitrates by, 640  
 Pyrophosphate-carbonate solution, 168  
 Pyrotannic detector for carbon monoxide, 115  
     scale reading, 115  
 Pyrrol, selenious acid by, 604
- Quinine** reagent, 514  
     phosphates by, 514  
 Quinalizarine, aluminum by, 270  
     beryllium by, 399  
 Quinine arsenomolybdate, 242  
     arsenic by, 242
- Range** of usefulness of nephelometer, 93  
 Reading errors in colorimetry, 79  
 Recording color, systems of, 72  
 Red lead, copper in, 149  
 Removal of interfering substances. *See* separation  
 Resorcinol, zinc by, 345  
 Rhenium, by potassium thiocyanate, 392  
     standard for, 393  
 Rhodamine B, tungsten by, 379  
 Rhodium, by stannous chloride, 423  
     in hydrochloric acid, 424  
     separation from other metals, 417  
 Rock, chromium in, 275  
     fluorides in, 572  
     iodine in, 560  
     manganese in, 335  
 Rosenheim-Schuster colorimeter, 20  
 Roulette comparator, 15  
 Rubber, sulfates in, 612  
     sulfur in, 612  
 Rubberized fabrics, copper in, 144  
 Ruthenium, separation from other metals, 416  
 Rutile, titanium in, 360
- Salicylic acid**, iron by, 301  
     titanium by, 364  
 Salicylsulfonic acid, iron by, 302  
 Saliva, thiocyanate in, 610  
 Salt brines. *See* brines  
 Salts, cobalt, nickel in, 315  
     iodized. *See* iodized salt  
     iron in, 308  
     perchlorates in, 590  
     thallium, thallium in, 209  
 Sand, platinum in, 413  
 Sanger-Black Gutzzeit apparatus, 237  
 Saturation, definition as applied to color, 6  
 Saybolt Universal chromometer, 28  
 Schiff's reagent, 545  
     bromides by, 544  
 Schreiner colorimeter, 42  
 S. D. C. colorimeter, 17  
 Sea water, ammonia in, 651  
     fluorides in, 582  
     hydrogen-ion concentration in, 687  
     iron in, 289  
     phosphates in, 509  
 Sealed tubes, 15  
 Seeds, iodine in, 558  
     phosphorus in, 489  
 Selenium, by hydroxylamine, 606  
     by mercurous chloride, 606  
     by potassium iodide, 604  
     by sodium hyposulfite, 605  
     distillation of, 233  
         apparatus for, 233  
     in pyrites, 606  
     in soil, 606  
     in sulfides, 606  
     in tissue, 606  
     in vegetable matter, 606  
     in water, 606  
     separation from other metals, 607  
     standards for, 604, 605  
 Selenious acid, by pyrrol, 604  
     by sodium hyposulfite, 605  
 Selenium sulfide paper, 184  
     mercury by, 184  
 Sensitivity of range of methods, error from variation in, 86  
 Separation, of aluminum from iron, 266

- Separation, of arsenic and selenium, 233  
  of antimony from other metals, 251  
  of beryllium from aluminum, 399  
  of bromides from iodides, 549  
  of bromides from reducing or oxidizing agents, 549  
  of chromium from manganese and iron, 335  
  of cobalt from copper, 323  
  of cobalt from copper and iron, 322  
  of cobalt from manganese, 323  
  of cobalt from nickel, 323  
  of fluorides from interfering substances, 577, 585  
    apparatus for, 578, 579, 586  
  of gold from platinum and palladium, 421  
  of iridium from other metals, 417  
  of manganese and iron from chromium, 335  
  of molybdenum from manganese and iron, 389  
  of osmium from other metals, 416  
  of palladium from gold and platinum, 421  
  of palladium from other metals, 417  
  of platinum from gold and palladium, 421  
  of platinum from other metals, 413  
  of potassium from phosphates and iron, 434  
  of rhodium from other metals, 417  
  of ruthenium from other metals, 416  
  of selenium from other metals, 607  
  of selenium and arsenic from soils, 233  
  of silica from iron and phosphates, 518  
  of silver from other metals, 411  
  of uranium from other metals, 395  
  of vanadium from other metals, 369  
Series of artificial standards. See standards  
Series of liquid standards, slide comparator for, 685  
Series of standards method, 1, 10  
  accuracy of, 74  
  calculation for, 97  
Serum, arsenic in, 228  
  calcium in, 461  
  hydrogen-ion concentration, 696, 711  
  magnesium in, 472, 476  
  phosphorus in, 487, 488, 513  
  potassium in, 434  
  sodium in, 441, 443  
  sulfates in, 625  
Sewage, ammonia in, 651, 652  
  chlorine in, 539  
  sulfides in, 594  
Silica, as silicomolybdate, 517  
  by reduction of, 522  
  colloidal, 519  
  present in silica determination, 518  
  in plasma, 524  
  in tissue, 523  
  iron in, 285  
  separation from iron and phosphates, 518  
  soluble, 518  
  standards for, 519, 522  
Silica and phosphorus, by ammonium molybdate, 520  
  by reduction, 521  
  in sugar, 521  
  standard for, 522  
Silicates. See also silica  
  fluorides in, 585  
Silicate minerals. See minerals  
Silicic acid, by pyrrol, 525  
Silver nitrate, apparatus for arsenic by, 248  
  arsenic by, 247  
Silver nitrate paper, arsenic by, 247  
Silver, as chloride, 409  
  as sulfide, 410  
  by reduction with hyposulfite, 411  
  separation from other metals, 411  
  standards for, 410, 412  
Silver chromate reagent, 535  
  chlorides by, 534, 536  
Silver nitrate, arsenic by, 247  
Silver ore, bismuth in, 213  
Slag, copper in, 144  
  fluorides in, 572  
  tungsten in, 377

- Slide comparator for liquid standards, 685, 712
- Sliding comparator, 16
- Soap solution, hydrogen-ion concentration in, 693
- Sodium, as complex sodium caesium bismuth nitrite, 444
- as complex uranyl sodium acetate, 439
- as pyroantimonate, 443
- by bismuth caesium nitrite, 444
- by eosin, 438
- in ash, 441
- in blood, 443, 444
- in feces, 445
- in gall, 445
- in milk, 445
- in organs, 445
- in plant tissue, 445
- in plasma, 444
- in serum, 441, 443
- in soil extracts, 442
- in urine, 440, 441, 444, 445
- standards for, 442, 443
- Sodium arsenite reagent, 175
- copper by, 174
- Sodium cobaltinitrite, 433
- potassium by, 432
- Sodium diethyldithiocarbamate, copper by, 164
- Sodium hypophosphite, 243
- arsenic by, 243
- Sodium peroxide fusion, titanium by, 362
- Sodium stannite, bismuth by, 223
- Sodium sulfocinoleate, calcium by, 453
- Sodium thiosulfate, molybdenum by, 392
- Sodium tungstate and titanous chloride, calcium by, 461
- Soil, citric acid extraction of, 491
- distillation of arsenic and selenium from, 233
- fluorides in, 574, 575
- hydrochloric acid extraction of, 491
- hydrogen-ion concentration in, 688
- iodine in, 560
- manganese in, 335
- nitrates in, 630, 631, 638, 641
- potassium in, 430
- selenium in, 606
- separation of selenium and arsenic from, 233
- Soil extracts, iron in, 301
- magnesium in, 476, 477
- molybdenum in, 387
- phosphorus in, 491, 492
- potassium in, 434
- preparation of, 491, 492
- sodium in, 442
- Solids, sulfides in, 594
- Soluble starch, preparation of dilute solution, 156
- Solutes present other than the test substance, error from, 83
- Sources of error, 74
- artificial standards, 69, 89
- dichromatism, 88
- dilution, 80
- importance of errors, general, 89
- impurity of reagents, 89
- in determination of hydrogen-ion concentration, 667
- in determination of platinum as iodide, 419
- mechanical, 75
- optical, 75
- readings, 79
- the individual operator, 85
- the presence of solutes other than the test substance, 83
- turbidity, 88
- variable light source, 77
- variable sensitivity of range, 86
- variable size of colloidal particles, 88
- varied temperature, 82
- varied time of standing, 82
- varied quantities of chemicals present, 82
- Spelter, aluminum in, 262
- Spinal fluid, arsenic in, 228
- lead in, 203
- Spot plate, 17
- Stammer colorimeter, 28, 51, 52
- Standard alkali solution, bottle for, 673
- Standard sunlight, 7
- Standard tubes, 13

- Standards, general, artificial liquid, 66  
cobalt-chromate-copper, 67, 68  
cobalt-iron-copper, 67, 68  
comparison of, 69  
permanganate-chromate, 68, 69  
errors of artificial, 69, 89  
glass, 32  
for hydrogen-ion determination, 686  
natural. Listed under substances determined
- Stannous chloride reagent, 457, 509  
arsenic by, 246
- Starch-iodine complex, gaseous oxygen  
by, 133  
apparatus for, 134
- Starch solution, preparation of, 156, 566,  
570
- Steel, ammonia in, 653  
carbon in, 101  
chromium in, 274  
cobalt in, 325  
copper in, 143  
manganese in, 334  
molybdenum in, 384, 385, 388, 391, 392  
nickel in, 315  
phosphorus in, 484, 485, 495, 496  
titanium in, 358  
tungsten in, 377  
vanadium in, 367, 368, 369, 371  
zinc in, 346
- Stools. See feces
- Strychnine iodomercurate, 185
- Strychnine reagent, 511, 513  
phosphates by, 511, 513  
phosphorus by, 513
- Strychnine sulfate reagent, 636  
mercury by, 185  
nitrates by, 636  
vanadium by, 371
- Sugar, arsenic in, 232  
decolorizing effect of vegetable carbon  
on, 26  
phosphorus in, 493  
solutions, apparatus for, 16  
hydrogen-ion concentration in, 688
- Sulfated fatty alcohols, hydrogen-ion concentration in, 693
- Sulfates, as alkali chromate, 626  
as barium sulfate  
nephelometrically, 618  
table for, 614, 615  
turbidimetrically, 611  
as benzidine sulfate, by furfural, 622  
by iodine and potassium iodide, 621  
diazotized and coupled, 623  
by lead sulfide, 626  
by liberation of chromate, 624  
by Tyndall effect, 615  
ethereal, 620  
in biological materials, 612  
in blood, 619, 620, 622  
in cement, 613  
in coal, 613  
in food, 612  
in milk, 622  
in rubber, 612  
in serum, 625  
in urine, 612, 620, 621, 625  
in water, 611, 618, 620  
inorganic, 619, 620  
neutral, 620  
standards for, 614, 615, 616, 621, 622,  
624, 626  
total, 619, 620
- Sulfide, colloidal, mercury as, 178
- Sulfides, arsenic in, 234  
as arsenious sulfide, 597  
by *p*-aminodimethylaniline, 593  
evolution of, 593, 598  
apparatus for, 599  
in feces, 594  
in foods, 594  
in gelatine, 594  
in iron, 594  
in mineral waters, 594  
in sewage, 594  
in solids, 594  
in water, 594, 596  
selenium in, 606  
standards for, 595, 597, 600
- Sulfonated oils. See oils
- Sulfur. See also sulfates  
by evolution as hydrogen sulfide, 598  
apparatus for, 599

- Sulfur, in petroleum distillates, 600  
  standard for, 601
- Sulfur dioxide, as sulfate, 627  
  by reduction of phosphomolybdic acid, 627  
  standard for, 628
- Sulfur monochloride, by ammonia, 603
- Sulfuric acid and iodine pentoxide, as carbon monoxide detector, 108
- Sunlight, standard, 7
- Sweepings, platinum in, 415
- Systems of color recording, 72
- Tag-Robinson colorimeter, 29
- Tailings, copper in, 144
- Tannic acid, molybdenum by, 389
- Tanning extracts, copper in, 151  
  iron in, 294
- Tanning liquors, chromium in, 278
- Tantalite, columbium in, 401
- Tartrazine, arsenic in, 232
- Tellurium, by mercurous chloride, 607
- Temperature, error from variation in, 82  
  measurement by color change, 22
- Test paper. See paper
- Test tubes, 10
- Tetramethyldiaminodiphenylmethane,  
  lead by, 200  
  manganese by, 342
- Textiles, chromium in, 275  
  copper in, 149  
  iron in, 295  
  manganese in, 338  
  mercury in, 182  
  rubberized. See rubberized fabrics
- Textile assistants, hydrogen-ion concentration in, 694
- Thallium, as phosphomolybdate, 210  
  as sulfide, 207  
  by liberation of iodine, 207  
  in grain, 209  
  in thallium salts, 209  
  in tissue, 208  
  in urine, 209  
  standards for, 207, 210, 211
- Thallous. See thallium
- Thiocyanate, ammonium, chlorates by, 589  
  cobalt by, 327  
  as copper pyridine thiocyanate, 608  
  as ferric thiocyanate, 608  
  in bile, 610  
  in blood, 609  
  in cerebrospinal fluid, 610  
  in feces, 609  
  in gastric juice, 610  
  in saliva, 610  
  in urine, 609  
  iron by, 283  
  potassium, bismuth by, 222  
  molybdenum by, 383  
  rhenium by, 392  
  tungsten by, 377  
  standards for, 608, 610
- Thiocyanate paper, 589
- Thiocyanic acid reagent for iron, 295
- Thioglycolic acid, iron by, 298
- Thiosalicylic acid, iron by, 300
- Thiourea, bismuth by, 220
- Three-cell colorimeter, 49
- Thymol, 363  
  titanium by, 363
- Thyroid, iodine in, 556
- Time of standing, error from variation in, 82
- Tin, as sulfide, 257  
  by ammonium molybdate, 256  
  by cacothelin, 258  
  standards for, 256, 257  
  stannic, 257. See also tin  
  stannous, 257
- Tin-base bearing metals, aluminum in, 262
- Tin ore, bismuth in, 213
- Tint photometers, 25
- Tintometer, Lovibond. See Lovibond tintometer
- Tissue, aluminum in, 262, 263  
  arsenic in, 228, 230, 235, 239, 240  
  bismuth in, 216  
  calcium and magnesium in, 453  
  chromium in, 280

- Tissue, copper in, 174  
   dry ashing, 262  
   gold in, 404  
   hydrogen-ion concentration in, 696  
   iron in, 293  
   lead in, 187, 203  
   manganese in, 337  
   phosphorus in, 489  
   selenium in, 606  
   silica in, 523  
   thallium in, 208  
   wet ashing, 263  
   zinc in, 346
- Titanium, by ammonium molybdate, 365  
   by dihydroxymaleic acid, 365  
   by gallic acid, 365  
   by hydrogen peroxide, 355  
   by salicylic acid, 364  
   by sodium peroxide fusion, 362  
   by thymol, 363  
   in cast iron, 358  
   in ferrosilicon, 359  
   in glass, 358  
   in iron ores, 360  
   in minerals, 363  
   in pig iron, 359  
   in pigments, 361  
   in rutile, 360  
   in silicate minerals, 356  
   in steel, 358  
   standards for, 361, 364, 365, 573
- Titanium trichloride, tungsten by, 375
- Titanous chloride reagent, 462  
   calcium as tungstate by, 461
- Tobacco, arsenic in, 231
- o*-Tolidine, chloramine by, 543  
   chlorine by, 538  
   gold by, 408  
   iodides and iodates by, 567  
   manganese by, 341  
   reagent, 538
- Trichloroacetic acid precipitation of proteins in blood, 487, 488
- Trichromatic colorimetry, 27
- Tubes, Eggertz, 13  
   Julian, 13  
   Nessler, 13, 14, 38, 42  
   sealed, 15  
   standard, 13  
   test, 10
- Tumors, chromium in, 280
- Tungsten, by hydroquinone, 378  
   by potassium thiocyanate and a reducing agent, 377  
   by rhodamine B, 379  
   by stannous chloride, 376  
   by titanium trichloride, 375  
   in alloys, 377  
   in filaments, 376  
   in films, 376  
   in iron, 377  
   in matte, 377  
   in minerals, 375  
   in ore, 377  
   in slags, 377  
   in steel, 377  
   molybdenum in, 384  
   standards for, 376, 379
- Tungsten and molybdenum, molybdenum in, 380, 381
- Tungstic acid, molybdenum in, 381, 384
- Tungstic acid reagent, 391  
   molybdenum by, 390
- Turbidimeter, Betz-Hellige, 617, 618  
   calibration curves for, 616
- Turbidimeter for series of standards, 10
- Turbidimetry, 90, 95
- Turbidity, as source of error, 88
- Turpentine, measurement of color, 22
- Tyndall effect, sulfates by, 615
- Typical sources of error. See sources of error
- Union colorimeter, 30
- Universal chromometer, Saybolt, 28
- Universal indicator, 685
- Uranium, by *o*-hydroxybenzoic acid, 394  
   by phenolic acids, 394  
   by potassium ferrocyanide, 395  
   in low grade uranium ore, 395  
   in organic matter, 395  
   separation from other metals, 395

- Uranium, standards for, 394, 397  
Uranyl acetate reagent, 440  
  sodium by, 439  
Urine, ammonia in, 652  
  bismuth in, 215, 217  
  calcium in, 449, 450, 451, 464  
  chlorides in, 533  
  hydrogen-ion concentration in, 695  
  iodine in, 558, 559, 563  
  iron in, 290, 299  
  lead in, 190, 191, 196, 203  
  magnesium in, 468, 472, 476  
  manganese in, 338  
  phosphorus in, 486, 490  
  sodium in, 440, 441, 444, 445  
  sulfates in, 612, 620, 621, 625  
  thallium in, 209  
  thiocyanate in, 609  
  zinc in, 351  
Urobilin, copper by, 172  
  zinc by, 352  
Vanadium, by ammonium molybdate, 372  
  by diphenylamine, 372  
  by hydrogen peroxide, titanium absent, 367  
    titanium present, 370  
  by phosphotungstic acid, 373  
  by strychnine, 371  
  in magnetite, 373  
  in minerals, 370, 371  
  in plant ash, 373  
  in steel, 367, 369, 371  
  separation from other metals, 369  
  standard for, 370  
Variation in color glasses, 33  
Vegetable oils, color of, apparatus for determining, 21  
Vegetables and vegetable matter, arsenic in, 235  
  copper in, 144, 149, 156  
  fluorides in, 585  
  iodine in, 553  
  manganese in, 337  
  selenium in, 606  
Vim-Sheftel colorimeter, 47  
Walpole technique, 11  
Warning against carbon monoxide, use of palladium chloride for, 112  
Water, aluminum in, 265  
  ammonia in, 649, 650  
  ammonia-free, 649  
  arsenic in, 235  
  calcium in, 459  
  calcium and magnesium in, 453  
  chlorine in, 539  
  copper in, 150, 151, 159  
  fluorides in, 574, 575, 581, 584, 585  
  hydrogen-ion concentration in, 687  
  iodides and iodates in, 567  
  iodine in, 552  
  iron in, 288, 289  
  lead in, 192, 193  
  manganese in, 335, 342  
  nitrates in, 629, 641  
  phosphorus in, 508  
  potassium in, 430  
  selenium in, 606  
  sulfates in, 611, 618, 620  
  sulfides in, 594, 596  
  sulfur in, 611  
Wedge-type colorimeter, 53, 55  
Wedge-type bi-colorimeter, 55  
Wesson colorimeter, 21, 63  
Wet ashing organic samples, 263, 497  
Whey, hydrogen-ion concentration in, 690, 692  
Wine, arsenic in, 247  
  copper in, 161  
  iron in, 294  
  phosphorus in, 490, 491  
Wool, dyed, chromium by, 280, 281  
Yeast, iron in, 311  
Yellow S, arsenic in, 232  
Yoe photoelectric colorimeter, 58, 59  
  light circuit for, 60  
  method of use of, 61  
  optical system of, 60  
  photocell circuit of, 58  
  tubes for, 61

- Zinc**, as sulfide, 346  
by diphenylthiocarbazone, 350  
by dithizone, 350  
by phosphomolybdotungstic acid, 352  
by potassium ferrieyanide, 348  
by resorcinol, 345  
by urobilin, 352  
in feces, 351  
in iron, 346  
in organic samples, 348, 349, 350, 353  
in steel, 346  
in tissue, 346  
in urine, 351  
standards for, 346, 348, 350, 352  
Zinc acetate solutions, 594  
Zinc mercurithiocyanate precipitates, copper by, 173  
Zinc oxide, iron in, 287  
Zirconium nitrate and 1,2,5,8-tetrahydroxyanthraquinone, fluorides by, 583  
Zirconium oxychloride and 1,2,4-trihydroxyanthraquinone, fluorides by, 582



## Some Recent Van Nostrand Text and Reference Books on Chemistry and Chemical Engineering

---

- Chemical Formulary.** *Edited by H. BENNETT.* Complete in every detail, this gives you not only ingredients and quantities of each to use, but it explains in full every step in the production of the finished material.  
 Vol. I.—Covers the standard formulas used in every industry. \$6.00  
 Vol. II.—Treats newer processes, new methods, new chemicals. \$6.00  
 Vol. III.—Contains the newest formulas, and the index to three volumes. \$6.00
- Standard Methods of Chemical Analysis.** *Edited by WILFRED W. SCOTT.* (IN COLLABORATION WITH RENOWNED SPECIALISTS.) Fourth Edition. Two Vols. (not sold separately). \$12.00
- Elements of Industrial Chemistry.** *By ALLEN ROGERS, Supervisor, Department of Chemistry, Pratt Institute, Brooklyn; Lecturer on Industrial Chemistry, Columbia University.* Second Edition. \$4.50
- A Manual of Industrial Chemistry.** *Edited by ALLEN ROGERS.* (IN COLLABORATION WITH EMINENT SPECIALISTS.) Fifth Edition. Two Vols. (not sold separately). \$13.00
- Unit Processes and Principles of Chemical Engineering.** *By JOHN C. OLSEN, PH.D., D.S.C., Professor of Chemical Engineering, Polytechnic Institute, Brooklyn, N. Y., Formerly, Sec. and Pres., Amer. Inst. Chem. Engrs.* (IN COLLABORATION WITH EMINENT SPECIALISTS.) \$5.00
- Van Nostrand's Chemical Annual.** Rewritten Seventh Issue, 1934. *Edited by JOHN C. OLSEN.* Flexible Fabrikoid. \$5.00
- Elements of Chemistry.** *By WILLIAM FOSTER, A.M., PH.D., Russell Wellmann Moore Professor of Chemistry, Princeton University.* Second Edition. \$3.25
- Inorganic Chemistry for Colleges.** *By WILLIAM FOSTER.* Second Edition. \$3.90
- A Treatise on Physical Chemistry.** *Edited by HUGH S. TAYLOR, D.S.C.* (Liverpool), *Professor of Physical Chemistry, Princeton University.* A Co-operative Effort by a Group of Physical Chemists. Second Edition. Two Volumes (not sold separately). \$15.00
- General Chemistry; Theoretical and Descriptive.** *By THOMAS P. MC CUTCHEON, PH.D., Professor of Inorganic Chemistry, University of Pennsylvania; HARRY SELTZ, PH.D., Associate Professor of Physical Chemistry, Carnegie Institute of Technology; and J. C. WARNER, Associate Professor of Electro-Chemistry, Carnegie Institute of Technology.* Second Edition. \$3.50
- Chemistry and Technology of Wines and Liquors.** *By KARL M. HERSTEIN, F.A.I.C., Consulting Chemist and THOMAS C. GREGORY, Consulting Chemist.* \$5.50
- Chemistry in Modern Life.** *By SVANTE A. ARRHENIUS, Late Director of the Nobel Institute, translated by CLIFFORD S. LEONARD, National Research Fellow.* (Library of Modern Sciences.) Full-page plates, diagrams. \$3.00
- Chemistry in the World's Work.** *By HARRISON E. HOWE, Editor, Industrial and Engineering Chemistry.* Pictures clearly, entertainingly and in non-technical language the relation of Chemistry to Industry. (Library of Modern Sciences.) \$3.00
- American Chemistry.** *By HARRISON HALE.* A record of achievement, the basis for future progress. Second edition. \$2.50
- The Law of Chemical Patents.** *By EDWARD THOMAS, of the New York and District of Columbia Bars, Member of N. Y. Patent Law Association, American Chemical Society, Associate Member of American Institute of Mining and Metallurgical Engineers.* \$6.00

*Van Nostrand books sent on approval to residents of the United States and Canada. General Catalog sent free on request.*

